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MOTOR IMAGERY AND MOTOR ILLUSION:

FROM PLASTICITY TO A TRANSLATIONAL APPROACH.

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CHAPTER 1. INTRODUCTION AND AIM OF THE STUDY

CHAPTER 1.1 MOTOR IMAGERY

1.1.1 DEFINITION

MI is commonly defined as the mental simulation of one's own performance without any associated overt movement[1], a dynamic state during which a subject mentally simulates a given action[2].

Motor imagination is part of a wider phenomenon, called motor representation, related to the will and the preparation of doing a movement. The concept of mental representation is deeply rooted in philosophy and in psychology, with a broad meaning and ancient origin: it can be considered as the mental process that represents external reality[3], a mental object with semantic properties. It describes the range of mental processes including perception to thought and memory. Motor representation is normally a non-conscious phenomenon, but it can be considered conscious in particular conditions: motor image is a conscious motor representation.

Action representation precedes execution, but it can be detached from it, it can exist on its own. It is important to understand the link that bonds (covert) REPRESENTATION of an action and (overt) EXECUTION of that action. This one-way relationship explains that an

execution is always preceded by a representation, while a covert action is not necessarily followed by an overt one[4].

Morris and colleagues[5] defined imagery as *"the creation or re-creation of an experience generated from memorial information involving quasi-sensorial, quasi-perceptual and quasi-affective characteristics, that is under the volitional control of the imagery, and which may occur in the absence of the real stimulus antecedents normally associated with the actual experience."*

MI is discernible in two types of imagery, based on the point of view of the subject. If the mental images are experienced as a spectator watching, from a third person view, a visual scene in which he/she makes an action, this is a visual or external imagery. If the subject imagines himself doing a movement from the first point of view, this will activate a more kinaesthetic way of imagination, based on the feelings associated with movement execution (ME), the so-called kinaesthetic or internal imagery. Indeed, motor imagery (MI) implies a representation of the body as an active being, interacting with the world and not a mere passive observation of the effect of forces on the external world.

At the beginning MI was studied in the psychological and in sport science fields and only by the late 1980s it was considered with a physiological approach. Cognitive neuroscience focused the attention on the processes underlying mental functioning. Mental imagery, the ability to generate a conscious image of the acting self, can be considered a neuronal process that involves specific brain structures[6].

Why did this happen so late? The main explanation is the complexity that concerns the assessment of MI. Imagined actions are private events, impossible to-be shared with the experimenter, while visual images are about external reality, common to other people.

However, it is possible to study MI objectively. Firstly, we have to take the assumption that MI is not a static event: it involves changes in the image over time, as the action goes on. So, mental action can be considered a simulation of the executed action[4].

Kinaesthetic imagery involves the sensations of how it feels to perform an action, including the force and effort perceived during movement, hence suggesting the body as a generator of forces[1]. Practically, these definitions suggest that MI is the prototypical form of motor simulation[4,7]. In his motor simulation theory, Jeannerod[4] postulated that represented actions might involve a large subset of the mechanisms that usually participate in the various stages of action generation, including motor execution. As a mentally simulated action, MI requires a sequence of cortical neuronal events, known as 'action plans', so that the temporal organization of a mentally imagined action should be similar to an executed one. So, there are three main concepts that describe dynamic changes in MI. The first one implies temporal characteristics of mental images and particularly, the **isochrony** of physical and mental performance of the same action. The very first researcher who talked about it has been Landauer[8], who showed that overt and implicit recitations of the alphabet took almost the same length of time; times for speaking aloud or thinking the same series of letters or numbers were similar. Since then it has been replicated as a tool to assess MI[9–11].

Another important issue is about the **programming rules**. Some variable, as the complexity of to-be imagined task or the accuracy of the movement could influence the duration of the mental action. This is again a feature shared with motor execution. Decety and Michel[12] showed that mental and actual temporal organization of movements were similar and involved the same planning program. ME follows Fitts' law[13] stating that movement time increased linearly as a function of task difficulty. This functional rule, that influences the

execution of a goal-directed action can be applied to a mental execution[14]. Interestingly it applies only to kinaesthetic imagery, while it did not to affect visual imagery[15].

Lastly, according to Jeannerod[16,17] and Johnson[18], the imagined movement obeys the **biomechanical constraints** of the represented movement. Imagined movements are organized following optimization principles. For example, in a grasping goal task, the spontaneously selected trajectory will avoid extreme movements, for an efficient fast and direct movement to reach and manipulate the object[19]. A similar pattern was found in MI movements[18,20]. This can be considered as a kind of implicit MI, a non-conscious mechanism used every day to prepare actions or a sort of simulation of the potential action without knowledge. Moreover, MI of a moving body segment took longer than mental representation of an object of another nature, with no anatomical constraints[21].

1.1.2 NEURAL BASIS OF MOTOR IMAGERY

With the assumption that imagining a movement is considered a simulation of the movement itself, we can expect to find an overlapping neural network activation, without the actual muscular activation[7,22–24].

Before the use of functional neuroimaging became common in the field of neuroscience, a lot of studies investigated imagination through autonomic changes. Indeed, producing an overt action would need some muscular strength that implies an adaptation of the organism. On the contrary, imagining a covert movement should not require this energy from the body, without producing any changes in the organism. However, adaptation to effort has a central component, in fact some modification in the autonomic system, assessed as

circulatory and respiratory response, were found during a MI task[25,26]. This led to the hypothesis that there is a central regulation that takes place before the metabolic urge[27,28]. Moreover, changes in the autonomic system are directly correlated with the difficulty of the to-be imagined task, as it becomes more complex more changes in autonomic system responses are recorded, because the resources to make that movement would require more energy. Autonomic activation during imagination is related to the central activation observed during the preparation of a movement. This brings us back to dualistic interpretation of MI, considered as a motor preparation by some and as a motor simulation by others. Jeannerod's view is that the activation of the autonomic system is the expression of a number of mechanisms that prepares the subject for a potential action. During MI the motor pathway is voluntary blocked by inhibitory mechanisms, while autonomic system is visible because of its non-voluntary control.

In the last decades, functional neuroimaging spread among neuroscience studies: the experimenter could 'see how' people do MI, taking the neural network under the spotlight, to have a direct measure of the brain activity.

The development of imaging techniques, in fact, changed dramatically the possibilities to study a living and working brain; particularly two techniques, the positron emission tomography (PET) and the functional MRI (fMRI). Thanks to their space resolution it was possible to improve the quality of the studies on MI[29].

The organization of motor activity is supposed to be based upon the utilization of information stored in the memory by the motor system in the form of multiple hierarchically organized representations of action. Grezes e Decety[22], suggested that MI recruits the

primary motor cortex (PMC), premotor cortex, supplementary motor area (SMA), anterior cingulate cortex, inferior and superior parietal lobules and the cerebellum (CB).

However, because of the low statistical power of fMRI and PET, linked to the often limited sample size[30] and the different tasks required, it is difficult to have generalized results. Depending on which part of the body is involved[31], the modality (visual MI or kinaesthetic MI)[32] and the complexity of the task (a single simple movement or a complex sequence of movements) a clear conclusion on the activated network might not be so easy.

In a single group of participants with high MI abilities, Guillot and colleagues[32] showed that visual imagery activated predominantly the visual pathways including the occipital regions and the precuneus, whereas kinaesthetic imagery involved mainly motor-associated structures and the inferior parietal lobule. Neuroimaging studies have also shown that the neural networks underlying MI differ as a function of both individual expertise level and imagery ability.

A recent meta-analysis by Hetu and colleagues[29] mapped involved regions and tried to assess the modulating effects of these variables. This interesting study revealed that during a generic MI a large fronto-parietal network is activated together with subcortical and cerebellar regions.

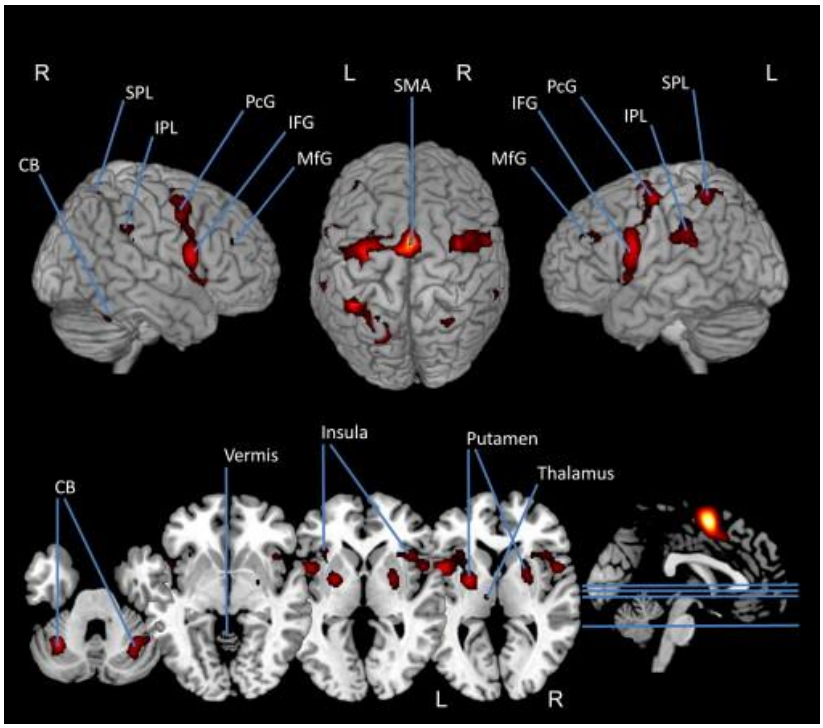

Figure 1. *Regions consistently activated during motor imagery[29].*

Frontal regions, particularly inferior frontal gyrus and SMA, are mainly associated with planning, preparation and execution of motor acts. Given the similar amount of time needed to perform an imagined and an actual movement[33], it is possible to think that we made similar steps in the brain. This reinforces the concept that MI, as motor execution implies a planning phase, before the simulation of the action. To confirm the importance of these brain regions, MI ability is lost in post-stroke patients with impaired frontal lobe functions, while it is preserved in post-stroke patients without premotor dysfunctions[18].

Parietal regions as inferior and superior parietal lobules, and supramarginal gyrus are consistently activated during MI. As a strategic sensory integration hub, parietal cortex is connected with different brain areas including premotor and primary motor cortex. Patients with apraxia after parietal damage even if they did not show any evident motor or sensory deficit[34], exhibited reduced ability to imitate actions on verbal command and some MI ability deficits[35]. Parietal cortex main role involves online control of movements when visual

information is available. However, during MI visual guidance is not needed, so the role of this part of the brain is probably not limited to this duty. The function of parietal cortex has been recently extended to higher cognitive and motor functions, as reaching[36], updating and maintaining postural representation of the upper limb[37], without visual input, particularly for SPL; or posterior parietal cortex (PPC) to code for the goal of actions[38,39].

In recent years a theoretical influential view suggests that MI is supported by motor system[23,40–42]. This is perfectly in line with our economic nature: is there a need for a differential system to simulate a movement when we have one made for moving? This assumption is also deeply rooted in our personal experience of MI: anyone who tries to imagine an action can "feel" the similitude.

It therefore seems that MI is not restricted to the simple activation of motor representations within the premotor and parietal cortex but rather, much like during motor execution, requires further processing of these representations. This may include motor initiation and/or motor selection, probably supported by the basal ganglia, and motor control supported by the CB.

Basal ganglia have been linked to the selection of motor programs during motor execution[43]. During motor behaviours, basal ganglia receive input from several cortical areas as well as from the thalamus. Different studies showed that basal ganglia are also consistently activated during MI. In line with the importance of the basal ganglia during MI, patients suffering from Parkinson's disease, which affects principally this region[44], show several impairments on MI tasks[45–47].

Cerebellum (CB): Various parts of the CB, as vermis and lobules VI and VII were consistently activated during MI. The CB, through its connections to the PPC, is involved in the execution of various types of movements[48]. Lesions to the CB are known to impair MI[49–51].

Controversy about the involvement of Primary Motor Cortex. Despite Motor Execution and MI relying on similar structures, PMC does not seem consistently activated during MI. PMC involvement is the object of a lasting controversy[24,42,52,53] in the neuroscience field: several authors suggested that an equivalent number of articles supported each position (PMC activation or not during MI). In his review, Hetu and collegues showed that only the 18% of the 122 articles considered, reported PMC activation during imagined movements. This does not mean that PMC is not involved at all, but it could simply mean that current fMRI and PET studies have not found any consistent activation. Otherwise, numerous transcranial magnetic stimulation (TMS) studies have provided strong evidence that MI can increase the excitability of the PMC (see[54,55] for reviews). This increase in excitability is assumed to be caused by activation of premotor[54] and/or parietal regions[56] which project to PMC. This situation is similar to the one regarding action observation/ mirror neuron system, where an increased excitability has been consistently recorded from PMC, even though there is little evidence that action observation directly activates this region by using neuroimaging techniques. One possible reason for increases in the PMC excitability without PMC BOLD signal increase during MI or action observation is that current fMRI/PET technology is not sensitive enough to pick up the subtle changes in excitatory/inhibitory processes that can be assessed with TMS. In fact, if any change in the PMC activity is actually produced during MI, it may be too rapidly suppressed for current fMRI/PET approaches to pick it up.

Additional evidence indicates that activation of PMC might be differentially influenced by MI instructions, MI ability, and motor expertise[57]. Taken together, the bulk of neuroimaging studies suggest that PMC is activated during MI – but weaker than during actual movement.

The inhibition paradox

As MI and ME shares the same brain networks, we can say that MI includes motor commands for muscle contractions, which, because of the nature itself of the imagined movement, are blocked at some level of the motor system by inhibitory mechanisms[58]. However, we have to take in consideration that the neural networks underlying these behaviours are not strictly identical. This is because when performing MI, participants are aware that movement will not be performed, and therefore that motor commands must be inhibited.

The brain needs to solve this paradox whereby it is required to issue the motor command needed for action when MI is performed, while concurrently issuing an inhibitory command when the person is moving during MI.

While mental operations of motor planning and programming are actually performed during MI, motor commands must be inhibited before being sent to peripheral effectors within the descending pathways. Excitability changes within motor cortical areas during MI, including changes in the activity of intracortical inhibitory or facilitatory circuits are analogous to those observed during motor preparation and execution[59,60]. It has been suggested that the Central Nervous System, manages to keep corticospinal facilitation below the motor threshold for activating the alpha motorneurons pool during MI[61].

Several studies showed that there were no changes in the H-reflex surface EMG traces evoked by electrical stimulation, and in combination with TMS, thus revealing that

corticospinal changes during MI occurred without any change in spinal excitability[62–64]. In summary, two theoretical models can explain the issue of inhibiting motor performance. First, assuming that MI results in a subliminal activity of the motor system. Therefore, if we consider MI as a subliminal motor command, it will not cause muscle activity and there is no need for active inhibition process. Second, the inhibition processes could occur at *every stage* of the represented action.

As we deep our attention on the second hypothesis, we have to consider that neuroimaging studies have failed to highlight specific neural structures mediating motor inhibition during MI, while TMS data support the idea of increased neuronal excitability and reduced intracortical inhibition within PMC during MI. A notable finding from neuroimaging research is that motor-related cerebral structures, like the CB and SMA might play a key role in motor output suppression during MI. Also, impaired sensory feedback integration following deafferentation or brain lesions around the primary somatosensory cortex results in weakened inhibition during MI, thus promoting the role of sensory sites in motor output suppression during MI. Therefore, inhibition during MI may be a functional process resulting from the specific contribution of neural sites usually dedicated to overt motor processing. This theoretical viewpoint might account for the fact that MI activates the motor system in a lesser extent to actual practice.

A recent study from Grosprêtre[65] demonstrates the presence of a subliminal motor output that activates low-threshold spinal structures, such as presynaptic interneurons, without modifying the excitability of alpha moto-neurons. The increase of the motor evoked potential (MEP) amplitude during MI gave a first hint of descending volleys along the corticospinal tract. The modulation of the H-reflex amplitude during MI, both when passively lengthening the muscle and when conditioning the spinal response, supported the activation

of presynaptic spinal interneurons when imagining. They suggest that MI activates both cortical and subcortical structures and that its impact on spinal networks depends, at least, on the excitability thresholds of the involved neuronal structures (interneurons and moto-neurons).

The enhancement of motor performance (strength gain, error decrease in motor sequence, etc..) after mental practice with MI has been mainly attributed to cortical changes[66]. The results of the study by Grosprêtre bring new evidence for a complementary hypothesis: in addition to neural plasticity at the cortical level, the reinforcement of synapse conductivity at the spinal level might participate in the benefits of MI practice.

1.1.3 MOTOR IMAGERY, LEARNING AND PLASTICITY

Systematic repetition of different states of action (physical and/or mental practice) has traditionally been approached as a way to improve performance. Through repetition, movements are executed faster, accurately, and effortlessly[67]. Two experimental paradigms are frequently used to study the neural processes underlying motor skill learning[68,69]: (1) motor sequence learning with the incremental acquisition of movements in a specific behaviour and (2) adaptation learning with the compensation for changes in the body or environmental dynamics. For both paradigms motor learning cannot be considered a linear process of performance improvement[70,71]. For instance, Doyon and Benali[68] highlighted the involvement of functional interactions between cortico-striatal and cortico-cerebellar brain systems during the early stages of motor learning, i.e., corresponding to the rapid

performance improvements consecutive to a single/a series of practice session(s). The automatization stage of motor learning, corresponding to slower performance improvements yielding to increased motor efficiency, involved to a greater extent the cortico-striatal system[72]. While learning stages differ in terms of behavioural /neurophysiological correlates, they commonly result from on line learning processes. Doyon and Benali also acknowledged the consolidation stage, characterized by delayed performance gains occurring after a latent period of approximately 6h, in the absence of additional practice. These can be summarized as *offline* learning processes, since they *indirectly* result from practice. Performance improvements consecutive to a night of sleep is a well-established correlate of offline learning[73].

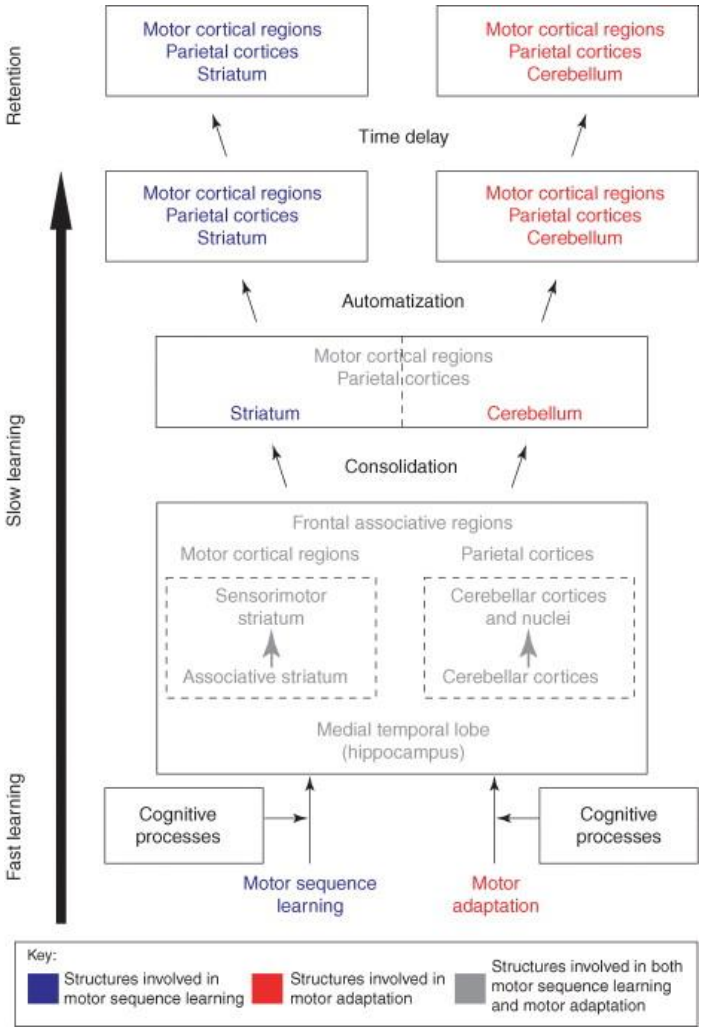

Figure 2. *Model describing the cerebral plasticity within the cortico–striatal and cortico–cerebellar systems during the course of learning a new sequence of movements (motor sequence learning) or to adapt to environmental perturbations (motor adaptation).*

As discussed earlier, there are a lot of studies that focused their attention to find and explain similarities between cortical activation during executed and imagined movements, and the functional equivalence between them. However, only little is known about how learning processes by execution or imagery work. Furthermore, it is unclear what are the similarities and differences of these ways of learning[74].

Learning is a result of direct and indirect interaction with the environment. This process leads to a transition from unskilled into skilled motor actions, resulting in refined planning and motor execution[75,76]. Actions can be overt (executed) or covert (imagined or observed)[7,77]. While 'real' movements imply both covert (planning) and overt (execution) stage of action, 'simulated' actions, as imagined ones, involve just the covert part. To this extent, any form of executed or simulated action is considered action that at some degree involves the motor action system. Given the principle of functional equivalence[16] and the simulation theory[4] , executed, imagined and observed actions are all suggested to be actions, as each draws on the same motor representation[74]. Accordingly, the repeated use of any of these states as mean of practice should lead to functional changes within motor action system to learning. The literature in sport psychology has provided relevant information about the positive effects of MI practice on motor performance[78]. Athletes and musicians extensively use mental practice, in addition to physical practice, to improve their dexterity[4]. Mental practice with MI improves several aspects of motor performance, such as muscle strength[79,80], movement speed, accuracy and variability[66,81–83].

In order to investigate the influence of mental practice on motor system, traditional research evaluated motor performance[78,84]. It is well known that mental practice (repeated MI) is more effective than a resting state without practice, but to a lesser extent than physical practice[85]. Moreover, combining mental and physical practice seems to be as effective as or superior to the only physical practice[86].

More recently neurophysiological studies used different tools as Non Invasive Brain Stimulation (TMS, transcranial Electric Stimulation (tES)) and fMRI to learn more about the adaptation of the brain as a result of practice. However, while considerable attention has been directed to comparing different states of actions, as the imagery and the execution of an action, few studies compare brain changes and learning after repeated actions using the different modalities (to imagine or to execute)[85,87]. Interestingly together with performance improvement, physical and mental practice seems to lead to similar plastic changes. Furthermore, individual motor imagery ability positively correlated with changes in movement performance induced by MI practice[85].

In the laboratory, after a paradigm of motor learning, it is possible to investigate consequent cortical neural plasticity, with the help of neurophysiological tools. One of the most common ways to detect excitability modulations includes testing associated cortical plasticity (changes in cortical excitability) with TMS. TMS is extensively used in cognitive neuroscience to determine the involvement of brain areas and the temporal specificity. It uses a magnetic field to activate neurons located a few centimetres under the coil. A brief stimulation over the cortical representation of a body part in PMC activates the corticospinal tract, and induces a response in the corresponding contralateral muscle. This response is called a motor-evoked potential (MEP). When placed over PMC, TMS elicited MEPs in the contralateral effector, a probe of corticospinal excitability. In the last few years a small

amount of studies assessed neural plasticity measuring cortical excitability before and after a MI practice[66,85,88]. Another popular method is highlighting changes in the effect of plasticity induced by NIBS techniques. Animal studies showed that motor learning leads to long-term potentiation (LTP) in the PMC[89]. This learning-induced LTP temporary occludes further potentiation, while enhancing long-term depression (LTD)[90–92]. Non-invasive techniques in humans also suggest that LTP-like plasticity is involved during motor learning. Paired-associative stimulation (PAS), consisting of transcranial magnetic stimulation of the PMC combined with electrical stimulation of the median nerve, can be used to measure LTP-like and LTD-like effects[93,94]. LTP-like effects are induced by an interstimulus interval of 25 ms (PAS25), while LTD-like effects by an interstimulus interval of 10 ms (PAS10). As in animals, a period of motor learning reversed or occluded LTP-like effects, whereas it either enhanced LTD-like effects or left them unchanged[95–97]. In a recent study it has been shown that[85] MI as well as ME practice produced a rate increase in the tested movement, together with an occlusion to further potentiation induced by LTP-like plasticity PAS protocol. These results reveal that, in addition to cortical reorganization, MI practice strengthened the synaptic connectivity[98].

1.1.4 AUGMENTED MOTOR IMAGERY

MI practice has a lot of advantages, as a unique opportunity to increase the number of repetitions in a safe and autonomous manner without excessive physical fatigue, but it also allows the mental rehearsal of motor tasks when and where the patient wants to practice and even in the case that the subject is not able to move. Yet, despite these obvious

advantages, mental practice is a complex mental process that is not readily amenable to be integrated into clinical habit.

Furthermore, as described above, studies using PET[99] and fMRI[100] observed small but distinct differences in intensity and location of brain activations between physical and MI training. These differences may arise from the absence of sensory feedback while imaging. When a MI training programme is followed by a physical practice session, the difference disappeared. This highlights the importance of additional sensory feedback for the consolidation of neural modifications induced by mental training. Moreover, as well described by Bassolino and colleagues[101], the shrinkage of hand cortical representation after immobilization is not compensate by MI. The group who performed mental practice during 10-hours arm immobilization in fact showed a reduction of corticomotor maps and excitability at the end of the immobilization period as the control group (rest during the 10-hours arm immobilization). One possible explanation given by the authors was that subjects did not perform MI. Still, MI participants showed a significant difference in the time required to mentally perform various types of grasping, being slower in the simulated execution of more complex tasks. This is in line with the previous studies, demonstrating that MI obeys the same physical constraints (e.g. Fitt's law on speed/accuracy trade-off[102]) applied to real movements[10,103], and suggests that they carefully performed the task during the immobilization period. Furthermore, such result confirms that a motor representation of the grasping movement subserving MI is still accessible even during arm inactivity. Thus, the inefficacy of MI to prevent corticomotor depression seems not dependent on a general impossibility to perform the task, but it could also be due to a less efficiency of MI in activating the motor cortex when the involved body part is prevented to move, as here because of immobilization[104]. These results support the hypothesis of an afference-

dependent relationship between MI practice and cortical plasticity. In a recent study, a Korean group[105] introduced a new approach using MI training combined with electromyography-triggered electric stimulation to improve paretic upper extremity motor function in patients with chronic stroke. They found behavioural improvements and an increased metabolism in the contralesional supplementary motor, precentral, and postcentral gyri. Then, the same paradigm was applied on healthy subjects,[106,107] showing that the administration of a sensory feedback during MI is able to induce a corticospinal facilitation similar to the one induced by voluntary movement and larger respect to MI or electrical stimulation alone (Figure 3).

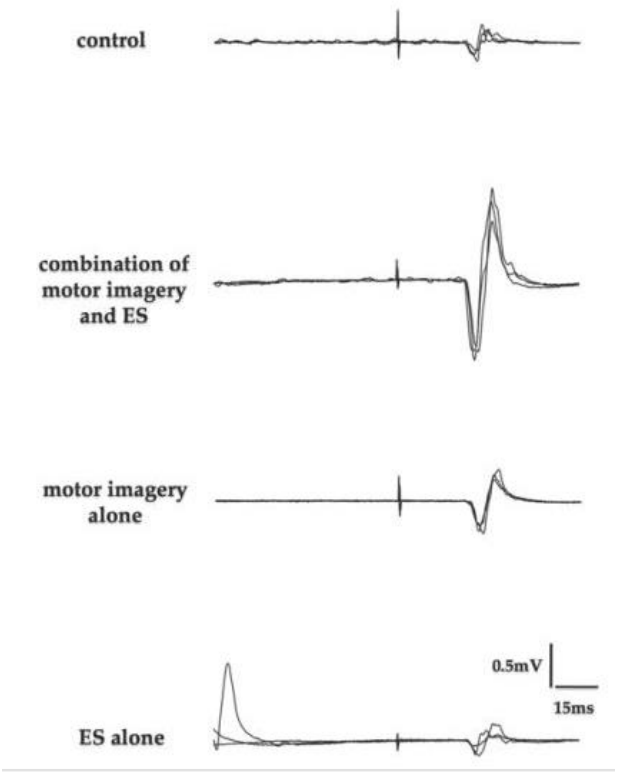

***Figure 3**. Representative Motor Evoked Potential (MEP) recording from thenar muscles during MI alone or the combination of MI and electrical stimulation*[106]

CHAPTER 1.2 MOVEMENT ILLUSION

When we decide to move, our brain has a constantly updated estimate of the configuration of the body's segments. Over the course of movement planning and execution, different multiple body state estimates are generated, combining current sensory input (primarily vision and proprioception) with the input that we will receive as a result from a motor command (forward internal models)[108].

Normally it is impossible to study what are the factors that influence body state estimate, but with an experimental paradigm, mirror illusion, it is possible to separate these intertwined parts. A mirror is placed sagittal midway between a participant's limbs. When the limb facing the reflective surface moves, it provides mirrored visual feedback of movement that is attributed to the limb hidden behind the mirror. If the two limbs moved asymmetrically relative to the mirror, the mirrored visual feedback about the hidden limb does not match the motor command sent to, or the proprioceptive feedback from, that limb.

***Figure 4**. Mirror box session*

This 'magical trick' allows an examination of how the brain estimates the state of the hidden limb. In addition to its usefulness as a tool to understand how the brain integrates vision,

proprioception, and predictions from the forward model to maintain body state estimates, the mirror illusion has also been used in the rehabilitation of sensorimotor deficits. Mirror illusions were first used as a tool to reduce phantom limb pain after amputation[109], from then on termed as mirror therapy. Phantom limb pain is the pain perceived by a region of the body no longer present. The illusion provided by the mirror is able to restore congruence between visual and proprioceptive inputs and to evoke the feeling that the amputated limb had been "resurrected" in some patients. Mirror illusions used for the treatment of phantom pain have been attributed to modulation of the sensory representation of the hidden limb by visual mirror feedback of the limb facing the mirror[110]. It was proposed by Harris[111] that one contributing factor in phantom pain, might be a mismatch between motor output and visual feedback from the arm and, at least in part, a response to the discrepancy between different senses such as vision and proprioception. If so, perhaps mirror visual feedback (MVF) acts by restoring the congruence between motor output and sensory input. Afterwards, this simple, cheap and less labour-intensive rehabilitation method was successfully used to recover motor function of the upper limb in patient with stroke[112]. In healthy subjects, the same mirror configuration can also be used to induce multiple motor and perceptual responses on the arm hidden behind the mirror[113]. For example, the mirror can lead to directional biases in reaching movements on the contralateral hand hidden behind the mirror[114] and could also enhance bimanual coordination[115]. Moreover, viewing the reflection of one's arm being passively moved induces consistent, vivid kinesthetic illusions of movement on the static arm hidden behind the mirror; this effect has been called the kinesthetic mirror illusion[116]. This illusion can be explained as resulting of the integration of conflicting visual and somatosensory inputs. The notion that powerful inter-sensory interactions can occur had already been evident from the work of Gestalt psychologists from

the early 20th century. A particularly compelling example was discovered by the pioneering experimental psychologist Rock and Victor[117]. They found that vision dominates touch and proprioception. Indeed, if an object was made to merely look larger than normal using a lens, while it was being palpated, it also was felt larger by subjects. Vision in most cases dominates touch[118].

However, the mechanism of rehabilitation based on the use of MVF (i.e., mirror therapy) is not fully understood[119]. There are several the theories, which can be classified into two general categories: a primary motor cortex mechanism and a mirror neuron system mechanism[119]. The PMC works with premotor areas to synthetize and to execute movement. Some studies showed cortical activity of PMC during visualization of the hand and its reflection[120].

The other explanation takes advantage of the discovery of Mirror neurons in monkeys by Rizzolatti and colleagues in 1990's. Such neurons were found in the frontal lobes as well as in the parietal lobes. Mirror neurons are also been shown within the frontotemporal region and the superior temporal gyrus, and are defined as bimodal neurons that fire when the animal performs a motor action, as well as when the animal observes another performing a similar motor action. In humans the concept of mirror neurons has been translated into that of a Mirror Neuron System.

Mirror Neuron System necessarily involve interactions between multiple modalities—vision, motor commands, and proprioception—which suggest that they might be involved in the efficacy of MVF in rehabilitation. This mismatching approach is lately more investigated and it is a promising therapy for a number of neurologic and orthopaedic pathologies.

CHAPTER 1.3 AIM OF THE STUDY

The aim of the studies carried on during my Ph.D. program, was to understand human's motor system response to different ways of moving without actual movement. This represented the conditio-sine-qua-non we could assess feasibility of a practical application of these techniques in the rehabilitative field. To this aim, we employed neurophysiological and behavioral methods to analyze mechanisms behind these techniques, i.e. MI and movement illusion, and their implications in learning and re-learning abilities.

Firstly, we decided to investigate the effects of different motor learning protocols, with and without actual movement, on behavioral performance and neurophysiological correlates. More in details, we trained three groups of participants with different training methods: motor execution, MI and an augmented version of MI (ES+MI). Previous studies[107,121] showed how peripheral nerve electrical stimulation (ES) can be the answer to the lack of peripheral afferent inputs during MI, where the combination of the two could enhance cortico-spinal excitability similarly to voluntary movement.

Here we analyzed whether a training with this combined technique was able to induce cortical plasticity as much as physical practice, and if the improvements in performance behavioral assessment were retained it normally happens after a real training.

Then, to better understand peripheral afferent stimulation role during MI, we focused on the physiological mechanisms of sensorimotor integration during MI. In particular, we assessed sensorimotor modulation at the starting point of an overt and of an imagined movement, using a TMS paradigm, short afferent inhibition (SAI)[122]. Moreover, if MI would have been able to produce a modulation, we wanted to see if the temporal and spatial characteristics where similar to the ones occurring during actual movements. On the second

part of this study we tried to assess if PMC was a site implied with sensorimotor modulation. In detail, we used transcranial Direct Current Stimulation (tDCS) to modify PMC excitability and we recorded any possible change in SAI modulation during MI or ME.

On the third study we wanted to deepen our knowledge of behavioral changes after motor learning induced by MI practice. To understand if, even without sensorial feedback, a mental motor learning followed the same pattern as motor execution, we recruited two groups of young students. One group executed a complex sequence of finger movements while the other group kinesthetically imagined a similar, but different, sequence. Our particular approach, with multiple days' assessments, allowed us to highlight possible differences through the entire acquisition process, from first improvements to consolidation and retention[68].

Lastly, we made a step forward a non-movement rehabilitative approach. The application of techniques alternative to overt movement in my professional field, as a physical therapist, is the ultimate goal of my three-year PhD program. Here we applied mirror illusion to Parkinson's disease (PD) patients, for the purpose of improve one of the most common motor symptoms that affect these patients, bradykinesia. Defined by slow movements and impaired ability to move the body swiftly on command, bradykinesia is usually more prominent in one side. We decided to reinvent a tool, the Mirror box, born to reduce pain in amputees and more recently used in post-stroke patients, to improve bradykinesia of the more affected hand of a group of PD patients. To fully explore the potential effects of movement illusion in rehabilitation, we did not limit ourselves to behavioral assessment, rather we used a neurophysiological tool, the TMS, to explore changes in PMC following Mirror box training.

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CHAPTER 2. INSTRUMENTS

2.1 GLOVE ANALYZER SYSTEM (GAS)

Wearable Engineered Glove

The engineered glove is built on a Lycra glove, easy to wear and not exerting constraints during the motor sequence execution (Figure 1). Five plates in gold are placed on the palmar surface of the distal phalanxes of the glove, in order to record the contact during opposition movements between thumb and another finger. Each plate is connected through its own wire and a specific connector in brass gilt to a bracelet in tissue with a Velcro closure. Tinsel interlaced with strands in Ag/Cu was used as wire.

The total distance from the plate to the bracelet is 30 cm. In the magnetic compatible version, a security resistor (12 kΩ) is placed at 1 cm far away the plate. From the bracelet, the five signals, corresponding to the five fingers, reach the acquisition board through a multipolar cable ending with a 9-pins RS-232 connector. Acquisition board is the USB-1208FS device (Measurement Computing).

Figure 1. *Sensor engineered glove*

Software

The software used for recording and analysis of the data was developed with the Microsoft Visual Studio 2008 package. GAS allows the setting of several types of protocols with different features (Figure 2). Three protocols are possible. The first is Self-Paced Mode (SPM). When SPM is set, subjects have to perform motor finger movements at their most comfortable rate. No external events pace movements. Similar to SPM is Maximum Speed (MS) protocol. Movements are not paced externally, but people are asked to move fingers at their maximum rate. On the other hand, in tasks with Metronome protocol (Metr) subjects are asked to execute the finger opposition movements using an external pace as a reference. Acoustic or visual events at fixed rate are used as metronome. Although subjects can tap in synchrony or in syncopation with respect to the cue, usually, the first condition is requested. As for other possibilities and variables, GAS allows to execute uni and bi-manual tasks and to select simple or complex sequences, complexity depending on both the length of and the finger order within the sequence. GAS implements algorithms to perform automatic analysis of parameters for describing finger opposition movements. Generally, analysis is performed on the sequences correctly executed within a task, but a specific tool allows to perform analysis also on incorrect touches. Mean value, Standard Deviation (SD) and Standard Error (SE) are automatically calculated.

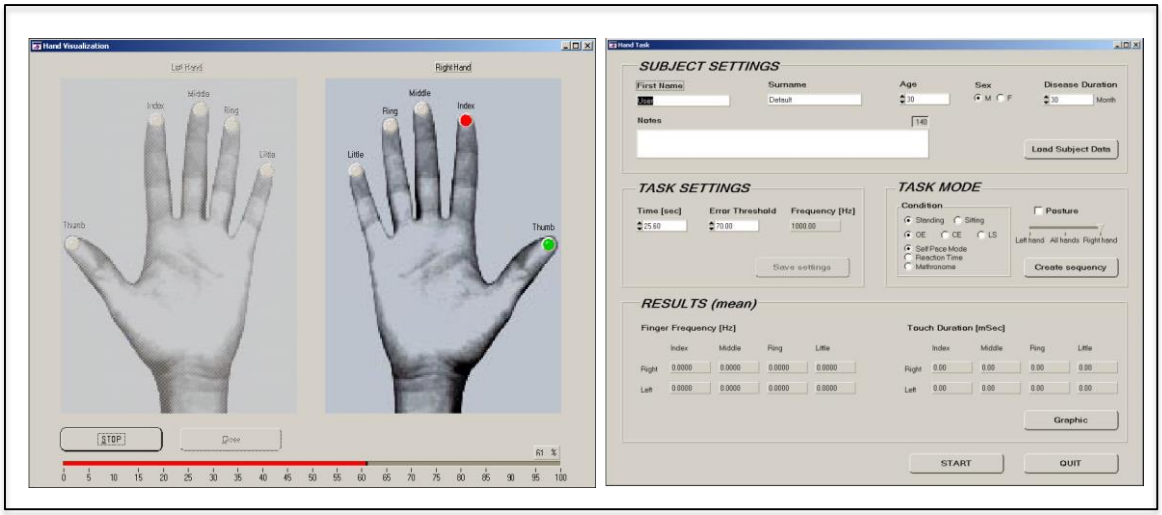

***Figure 2**. Graphic User Interface of GAS.*

Parameters

In order to describe spatial and timing accuracy for finger opposition movements, several parameters are used. In particular, we considered primary and secondary parameters. The first are directly calculated on the raw data acquired during execution of motor finger sequences. The second are obtained as combination (ratio, percentage, etc.) of primary parameters.

Spatial accuracy

- *Touch Duration (TD)*: contact time between thumb and another finger. TD is thought as the portion of a movement implicated in finger discrimination. Values are expressed in milliseconds. (Primary parameter)
- *Inter Tapping Interval (ITI)*: time between the end of one touch and the beginning of the successive one in the sequence. ITI reflects aspects related to the velocity movement. Values are expressed in milliseconds. (Primary parameter)
- *Movement Rate (MR)*: velocity of the movement expressed in Hz. MR is calculated as ratio 1/(TD+ITI). (Secondary parameter)

- *Ratio TD/ITI*: adimensional parameter that expresses the relative quantity of the movement spent in finger discrimination. Values are independent from performed Movement Rate. (Secondary parameter)
- *Number of Correct Sequences*: number of sequences correctly executed during the execution of a task. Number depends on the Movement Rate, both internally and externally paced, and on the number of error. (Primary parameter)
- *Error Number*: absolute number of errors performed during the execution of a task. For sequences involving only one finger, no errors are computed. Errors take into account skipping a finger, a double touch to the same finger and a touch of the thumb to two or more fingers at once. Values are adimensional. (Primary parameter)

  Eventually it is possible to express EN as:

  - *Error Number Percentage*: the absolute number of errors divided by number of correct sequences.
  - *Normalized Error Number*: the absolute number of errors divided by performed Movement Rate.

Temporal accuracy

When Metronome protocols are used, GAS executes also temporal accuracy analysis. Time between two consecutive events can be divided in phases. Movements with phase shifts from −0.5 to −0.25 and 0.25 to 0.5 cycle were defined as “syncopated” or anti-phase movements. Those with phase shifts from −0.25 to 0.25 cycle were defined as “synchronized” or in-phase movements.

We computed the temporal accuracy parameters by normalizing event-movement onset with respect to event occurrence.

- *Timing Error (TE)*: time between the touch onset and the corresponding acoustic or visual cue. When the touch precedes the metronome event, TE is negative; when the touch follows the metronome event, TE is positive. In order to describe the lack of synchronization independently from touches in advance or delay, TE absolute (TE_{abs}) can be used. Values are expressed in milliseconds. (Primary parameter)
- *Advance Percentage & Delay Percentage*: the number of finger movements with negative onset (from -0.5 to 0 of a cycle) and positive onsets (from 0 to 0.5 of a cycle) with respect to the event occurrence. Advance and Delay are complementary parameters. Values are adimensional. (Primary parameter)

When bimanual tasks are performed and the sequence selected for both hands is the same, temporal accuracy also involves parameters that describe performances of synchronization of dominant hand respect to non-dominant hand.

- *Inter Hand Interval Onset*: the temporal value between the touch onset occurring in non-dominant hand and the corresponding touch in dominant hand. Values are expressed in milliseconds. (Primary parameter)

  Inter Hand Interval Offset: the temporal value between the touch offset occurring in non-dominant hand and the corresponding touch in dominant hand. Values are expressed in milliseconds. (Primary parameter)

Usually the analysis of bimanual tasks adopts absolute values of inter hand interval.

NON INVASIVE BRAIN STIMULATION (NIBS) TECHNIQUES

From the neurophysiological point of view, we based our experiments on the use of NIBS techniques. Over the last two decades, an increasing number of researchers have applied a variety of NIBS techniques to probe plasticity processes, changes in cortical excitability, in the PMC. Interference or improvements in behavioural and motor learning tasks can be used as a possible assessment of the impact of NIBS protocols. Widely used NIBS protocols are, Paired Associative Stimulation (PAS) and transcranial direct current stimulation (tDCS). During my PhD program I used these two techniques to better understand the effects of different ways of training and moving on cortical plasticity and circuits.

2.2 TRANSCRANIAL MAGNETIC STIMULATION (TMS)

Overview

Investigations on conduction of the nerve impulses from the motor cortex to the spinal cord offered in the last decades clinical and experimental advantages, such as the possibility to validate the integrity of motor nervous pathways and to obtain functional maps of brain areas.

Severe discomfort brought, for example, by Transcranial Electrical Stimulation, induced many researchers to test new techniques, such as TMS. The first successful TMS study using the principle of electromagnetic induction applied to the brain was performed in 1985 by Barker et al. In particular, TMS uses a coil of wire encased in plastic. When it is energized by the rapid discharge of a large capacitor, a rapidly changing current occurs, producing a magnetic field oriented orthogonally to the plane of the coil. When that coil is placed on the

head, the magnetic field, by passing through the insulating tissues of the scalp and skull, induces an oppositely directed current in the brain that flows tangentially to the skull and performs two consecutive effects. Firstly, the pulses of current induced in the structure of the brain activates neurons and interneurons placed horizontally to the cortex surface (Figure 3). Secondly, these excited nervous cells stimulate the cortico-spinal neurons trough a transynaptic activation. For these reasons TMS can be used to investigate both the nervous conduction along cortico-spinal pathways and the state of excitability of the motor cortex (Figure 4).

Therefore, when we are choosing the coil we must to take into account several technical requirements in order to satisfy our purposes. In fact, coils can differ in a lot of features, concerning magnetic field, current rise time, wave form, type of material, or biophysical characteristics of the pulse. The geometry of the coil is also important and several shapes are possible:

- round coil (original type of TMS coil)
- figure-of-eight coil (i.e. butterfly coil): very focal pattern of activation
- double-cone coil: conforms to shape of head; useful for deeper stimulation
- H-coil (Deep TMS): currently used in a clinical trial for the treatment of patients suffering from clinical depression

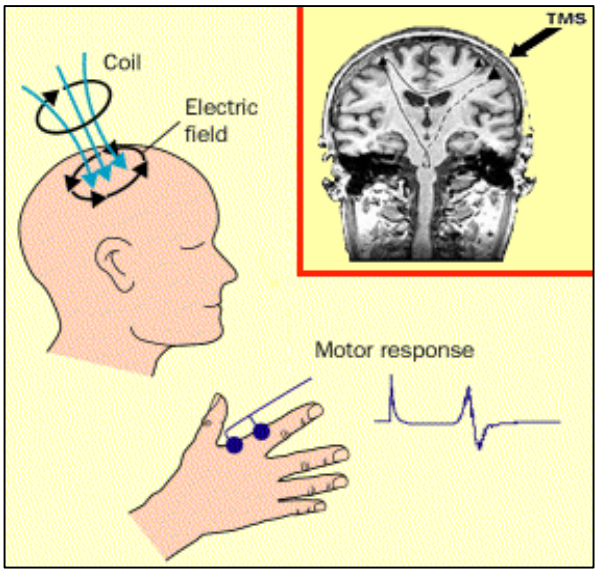

Figure 3. *When coil is placed on the head, the magnetic field, by passing through the insulating tissues of the scalp and skull, induces an oppositely directed current in the brain that flows tangentially with respect to skull and performs two consecutive effects. Motor Evoked Potential (MEP) can be recorded from the finger corresponding to motor stimulated brain area.*

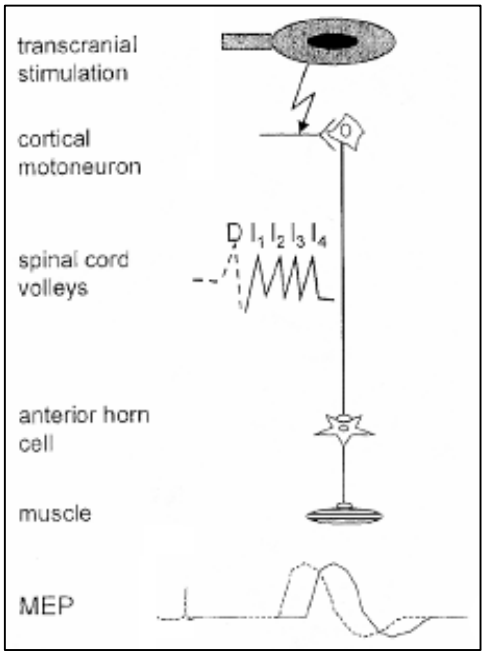

Figure 4. *The pulses of current, induced in the structure of the brain, activates neurons and interneurons, placed horizontally to the cortex. The excited neurons and interneurons stimulate the cortico-spinal tract trough a transynaptic activation and MEP recording is possible.*

In general TMS is a noninvasive method to excite neurons in the brain. Indeed, the risk of seizure increases, even if it remains still low, when TMS is delivered in a repetitive mode, at high rates (>5Hz) and intensity. Thus, brain activity can be triggered with minimal discomfort, and the functionality of the circuitry and connectivity of the brain can be studied. In fact, by stimulating different points of the cerebral cortex and recording responses from muscles, functional brain maps can be obtained.

In Single or Paired Pulse TMS, the pulse is delivered as a single or coupled event and different effects, such as changes in brain activity, can be detected. As PET and fMRI have shown, these effects do not outlast the period of stimulation[1]. When TMS is applied in the primary motor cortex, motor-evoked potential (MEP) are produced and recorded with electromyography (EMG) from muscles corresponding to the brain stimulation site. Stimulations on the occipital cortex, instead, produce flashes of light (phosphenes) detected by the subject. However, in most other areas of the cortex the stimulation effects are not consciously experienced, although behavior alteration may be produced (e.g. slower reaction time on a cognitive task). Recording of MEP amplitude, MEP latency, and central motor conduction time are used to study corticomotor function in neurological diseases as stroke, spinal cord injury, multiple sclerosis and motor neuron disease. TMS is also used to measure activity and function of specific human brain circuits. Different paradigms produce distinct inhibitory or facilitatory responses in cortical activity[2].

TMS can be used alone, with simple or double pulses, as a cortical circuits assessment tool, or it can be used in association with an electrical peripheral stimulation. In this second way of use, one of the most widely accepted protocols involves median nerve stimulation

followed by a single TMS pulse to the motor cortex approximately 20 ms later[3]. Peripheral nerve stimulation reduces the amplitude of MEPs when the two inputs are timed to nearly coincide within the motor cortex, delivering a TMS pulse ∼2–8 ms after the arrival of the afferent volley in somatosensory cortex (i.e., corresponding to the N20 somatosensory evoked potential).

SAI appears to originate within the sensorimotor cortices since it is not observed with transcranial electrical stimulation and there is a concomitant suppression of late indirect waves[3,4] with no impact on spinal excitability as measured by F waves[3,5,6]. SAI is found in both homo- and heterotopic muscles after stimulation of the mixed median nerve at the wrist[7]. In contrast, SAI evoked by digital nerve stimulation demonstrates a somatotopic distribution such that SAI is greater (i.e., more inhibition) and occurs at an earlier latency in muscles that are in closer proximity to the stimulating electrode[5]. SAI is modulated during specific phases of movement[8–10], indicating its use in studying motor control. SAI is reduced and/or abolished in Alzheimer's disease[11] and Parkinson's disease[12,13], particularly in those presenting with mild cognitive impairment[14] or dementia[15]. The role of cholinergic activity in cognitive functions has been well established[16]. The presence of a relationship between cognitive deficits and reduced SAI response supports the notion that SAI is a direct measure of cholinergic activity. Pharmacological studies demonstrate that SAI is abolished in the presence of lorazepam and scopolamine[4,17] and is increased with diazepam[17], indicating the influence of different $GABA_A$ subunits and acetylcholine in the genesis and/or maintenance of this circuit.

Another way of using the combination of a TMS central stimulation and a peripheral Electrical Stimulation, is in order to induce long time changes in cortical excitability.

A well-known protocol that expects low-frequency repetitive median nerve stimulation paired with TMS over the contralateral motor cortex, in fact, leads to cortical excitability changes in human primary motor cortex[18]

It was hypothesized that lasting excitability changes may be induced in the motor cortex by pairing median nerve stimulation with TMS over the motor cortex, because the magnetic stimulation excites the pyramidal cell indirectly through the axons of excitatory interneurons and because somatosensory inputs converge on pyramidal cells located in the motor cortex. This approach prompted the development of the PAS method, a paradigm consisting of low-frequency repetitive stimulation of the median nerve (typically 90–200 stimuli) combined with time locked TMS over the contralateral motor cortex. PAS with the interval between the two associative stimuli set at 25 ms (PAS25) led to a strong facilitation of MEPs, whereas inhibition occurred when the interval between peripheral and cortical stimulation was reduced to about 10–15 ms[18,19]. This bidirectional PAS-induced plasticity is reminiscent of what is observed in experimental models of associative long-term synaptic plasticity, i.e., long-term potentiation, LTP, and long-term depression, LTD[18–21]. In addition, PAS-induced excitability changes followed the rules of homeostatic plasticity[22].

On motor corticospinal output, the effects of PAS are rapid (within 30 min), persistent (>30–60 min duration), reversible and topographically specific. By investigating the effects of PAS on somatosensory and auditory evoked potentials, it has been shown that similar effects are present in the somatosensory and auditory cortices as in the motor cortex. As a general rule, the interval separating two consecutive pairs of conditioning-test stimuli (PAS frequency) can affect the pattern of motor cortical excitability changes. Usually, PAS is delivered at a relatively low frequency (0.01–0.25 Hz)[18,23,24], but some authors showed long-lasting changes in motor cortex excitability following PAS25 applied at 5 Hz[25].

Pharmacological studies have demonstrated the involvement of NMDA receptors in PAS25[19,20]. PAS25 does not change the SICI[25–27], suggesting that PAS does not influence inhibition mediated by the GABAA receptor, whereas it increases the duration of the CSP recorded from a contracting muscle[18,28,29], suggesting an influence on GABAB receptor-mediated inhibitory circuits. LTP-like effects of PAS25 were also not associated with enhanced intracortical glutamatergic transmission as revealed by lack of ICF changes[25]. In sum, PAS25 does not affect short-latency intracortical circuits[30], but, as demonstrated by Kujirai and colleagues[27], those recruited at long latencies are facilitated by PAS.

2.3 TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS)

tDCS was re-introduced as a NIBS technique applicable in humans approximately 15 years ago[31,32]. Its principal mechanism of action is a subthreshold modulation of neuronal membrane potentials, which alters cortical excitability and activity dependent on the current flow direction through the target neurons[33]. Other biological effects of the electric field are also likely relevant (changes in neurotransmitters, effects on glial cells and on microvessels, modulation of inflammatory processes). In analogy to pharmacological neuromodulators, tDCS does not induce activity in resting neuronal networks, but modulates spontaneous neuronal activity[34]. Consequently, the amount and direction of effects critically depend on the previous physiological state of the target neural structures[35,36] . In this sense, tDCS or transcranial alternating current stimulation (tACS) represents neuromodulatory techniques. It does not induce massive synchronized discharge of action potentials as TMS does. It never induces muscle contractions when applied above the motor cortex. During stimulation, the continuous current induces changes in membrane polarity by modulating the conductivity of sodium and calcic channels. After stimulation, according to the direction of the current, it

can induce excitatory (anodal tDCS), or inhibitory (cathodal tDCS) after-effects, via a LTP/LTD-type synaptic plasticity mechanism, NMDA receptor-dependent.

During tDCS), both anodal tDCS and cathodal tDCS, the delivered current is direct and monopolar. At the start and end of the stimulation, the current is gradually increased/decreased until the desired level of intensity (fade-in/fade-out periods). Because of single neuron registrations, we know that the application of a direct current can depolarize (anodal stimulation) or hyperpolarize (cathodal stimulation) the neuronal membrane potential, which enhances or diminishes the neuronal firing rate[37]. Thus, at a cellular level, the direct current impacts the membrane excitability in the opposite way depending on the stimulation polarity. Furthermore, these polarization effects persist beyond the tDCS period[38], and the after-effects involve the participation of glutamatergic N-methyl-d-aspartate receptors[39] and therefore long-term potentiation-like mechanisms. The amount of neuronal Ca2+ influx caused by the stimulation protocol has been proposed as a crucial factor in explaining nonlinear tDCS effects[40–42]. A modest and prolonged postsynaptic increase of Ca2+ levels leads to long-term depression, and a moderate increase induces no synaptic modulation whereas a brief but large increase of Ca2+ triggers long-term potentiation-like effects[43]. The no man's land explanation suggests that both the intensity and duration of tDCS carry significant biological information.

tDCS can be applied on human scalp with various aims, for example, for enhancing skill learning in spatial and verbal working memories[44,45], language acquisition[46] and motor skills development[47,48]. For a review of tDCS enhancements[49].

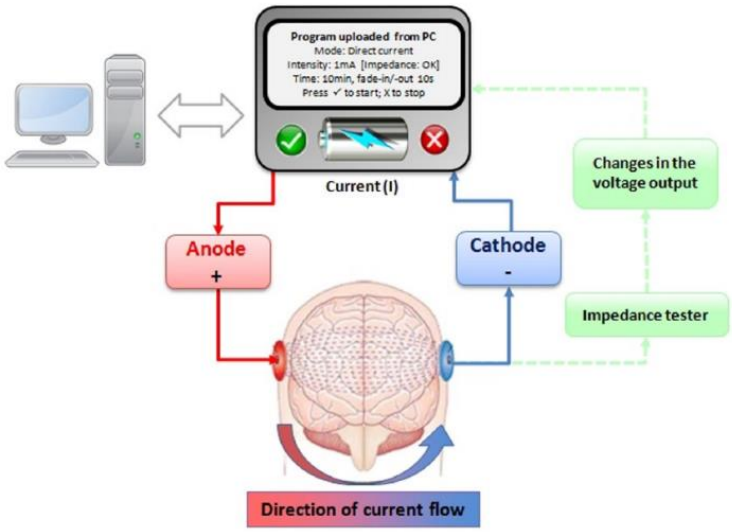

Figure5. *Schematization of the transcranial electrical stimulation (tES) stimulating device. Ferto-min17*

tDCS consists in applying continuous electrical current stimulation on the scalp between 2 non-metallic electrodes surrounded by a sponge soaked in NaCL solution. A continuous constant low-intensity current, from 1 to 2 mA, is applied during 10 to 20 minutes via a small galvanic stimulator, easy to transport and which can be pre-programmed in advance[32]. Once the region of interest is decided, commonly localized with 10:20 EEG system[50], the stimulating electrode is placed on the scalp. The reference electrode is commonly placed opposite the target electrode, or on the contralateral supraorbital region. Finally, they are secured with hypoallergenic tape or rubber bands. The mechanism of action for tDCS is very well-known[34] and this technique is well-tolerated. There is a slight tingling sensation under the active electrode upon stimulation, which usually disappears after a few minutes. This particularity makes it an excellent placebo. No severe adverse event has been reported with this technique, when respecting the usual recommended usage parameters, i.e. 1 to 2 mA intensity, with stimulation duration < 25 minutes. If these parameters are not respected (stimulation duration > 25 minutes and stimulation intensity > 2 mA or using water instead

of NaCL), it can lead to a transient local irritation under the active electrode. The most important safety parameter in fact, is current density. Current density, as used in the literature, indicates the average current density (in A/m^2) at the electrode calculated by taking the applied current to a given electrode and dividing by electrode area. Average current density is not necessarily indicative of peak current density at the electrode (which may be concentrated at edges or spots) or in the brain (which depends on many other factors namely head anatomy)[51,52].

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CHAPTER 3. MOTOR IMAGERY: NEUROPHYSIOLOGICAL STUDIES

3.1 PROVISION OF SOMATOSENSORY INPUTS DURING MOTOR IMAGERY ENHANCES LEARNING-INDUCED PLASTICITY IN HUMAN MOTOR CORTEX

3.1.1 INTRODUCTION

Motor practice leads to acquisition or improvement of kinetic skills. With physical exercise, movements become faster, more accurate and effortless (i.e., motor learning)[1]. Motor learning through repetition of a movement leads to a long-term potentiation (LTP) of the primary motor cortex (PMC) in humans as in animals[2]. This phenomenon of LTP leads to a temporary occlusion of PMC to further potentiation, according to the concept of homeostatic plasticity[3–6]. Evidence in the literature suggests that in humans, the occlusion of LTP-like plasticity after learning, indicative of how much LTP was used to learn, is essential for retention[7,8].

Paired associative stimulation (PAS) is a transcranial magnetic stimulation (TMS) protocol able to induce LTP-like effects in PMC[9,10]. PAS25 consists of pairs of electrical stimulation of the median nerve, followed by TMS of the hand area of the contralateral PMC at an interstimulus interval of 25 ms. PAS25 leads to an LTP-like long-lasting increase in MEP amplitude of a median nerve innervated hand muscle[11].

Noteworthy, other TMS techniques are able to induce LTP-like plasticity in PMC, such as repetitive TMS or theta-burst stimulation. These techniques imply the application of train of TMS stimuli over PMC in a regular fashion (trains of TMS stimuli repeated at frequencies higher than 1Hz) or in a patterned fashion (50-Hz triplets repeated at 5 Hz). PAS on one side, and theta burst stimulation and repetitive TMS on the other, differ particularly in the role of sensory input, which is relevant in PAS, but absent in theta burst stimulation and repetitive TMS[12]. Indeed, PAS25 is a plasticity induction protocol that is dependent on sensory afferent stimulation. For this reason, PAS25-induced plasticity shares similarities with experimental protocols inducing synaptic "spike timing-dependent plasticity" in vitro and in vivo[12,13]. Thus, PAS25 has been showed to be the most appropriate protocol to use when testing PMC plasticity induced by sensory afferent stimuli or by inputs to PMC coming from neural structures influencing sensory processing, including cerebellum[14].

Motor learning is also achievable without moving, with internal simulation of the movement, namely motor imagery (MI). Actual and imagined movements trigger similar motor representation and share similar brain substrates[15,16]. Particularly, imagery-related activity is in general more closely related to instruction-related activity (motor planning phase) than to motor execution-related activity[17]. In a recent study we investigated PMC plasticity induced by physical or motor imagery practice[18]. Both protocols were able to improve motor performance, even if improvement was greater after physical practice than after motor imagery practice. Furthermore, an increase in PMC cortical excitability was present after physical practice, but not after motor imagery practice. Finally, both protocols led to the development of neuroplasticity, as they affected the PAS25- induced plasticity in PMC, but observed effects after physical practice were stronger than after motor imagery practice. We explained these findings as related to different sensorimotor mechanisms operating during

the two training methods. Indeed, one of the main differences between physical and motor imagery practice is the lack of somatosensory afferent inputs in the imagined movements.

It has been recently demonstrated that the association of motor imagery and peripheral nerve electrical stimulation could enhance cortico-spinal excitability during MI practice, to a larger extent with respect to peripheral nerve electrical stimulation or MI alone[19,20]. Particularly, the combination of the activation of the internal model of motor commands, due to the MI, and the external activation of afferent input, given by peripheral nerve electrical stimulation led to a similar increase of the cortico-spinal excitability as real movement.

Here we explored whether a training session based on the combination of motor imagery and peripheral nerve electrical stimulation leads to (i) occlusion of LTP-like plasticity in PMC and (ii) retention of skill learning likewise physical practice. To test for LTP-like plasticity we adopted the PAS 25 protocol, since, as stated above, it is the most suitable non-invasive brain stimulation protocol to test for plasticity induced by sensory stimulation.

To this aim, we assessed LTP-like plasticity in PMC and retention of motor skill induced by motor learning through (i) motor imagery combined with peripheral nerve electrical stimulation; (ii) motor imagery alone and (iii) physical practice training sessions. We hypothesized that learning through motor imagery combined with peripheral nerve electrical stimulation would be more efficient than MI alone in inducing occlusion of LTP-like plasticity and retention of motor skills, which is known to be dependent on the occlusion of LTP-like plasticity.

3.1.2 MATERIALS AND METHODS

Subjects

All participants were in good health, without any nervous, muscular, orthopaedic or cognitive disorders. Right arm dominance was determined by means of the Edinburgh Handedness inventory[21]. Participants' general motor imagery ability was evaluated by means of the Italian version of the Movement Imagery Questionnaire (MIQ-R)[22]. The MIQ-R is an 8-item self-report questionnaire, in which participants rated the vividness of their mental representations using two 7-point scales, associated to kinaesthetic and visual imagery: the score ''7'' means ''really easy to feel/see'', whereas the score ''1'' corresponds to ''really difficult to feel/see'' (best score = 56, worst score = 8). All participants showed good motor imagery abilities (mean ± SD: 45.81 ± 4.9). The experimental protocol was approved by the ethics committee of the University of Genoa and was carried out in agreement with legal requirements and international norms (Declaration of Helsinki, 1964). All subjects gave informed consent for participation in the study.

Experimental design

Forty-four right-handed subjects (mean age 24.97 ±4.99, 18 males) participated in this study. All participants took part to a first experimental session (Day 0) designed to test the effect of PAS25 on motor evoked potentials (MEPs). Subjects were admitted to the subsequent experimental sessions if, in the first session, PAS25 had led to a significant increase of MEPs amplitudes. To control for it, we recorded twenty MEPs from the target muscle before and after a 30 minutes -PAS25 protocol on the homologous motor cortex. MEPs data collected before and after PAS 25 were compared by means of a paired Student t test. Only participants in which statistical analysis between MEPs before and after PAS25 yielded a statistically significant increase at a $p<0.05$ were admitted to the subsequent sessions.

Of 44 subjects, 36 fulfilled this criterion and were randomly divided in three groups for participating to the next experimental sessions (Day 1-Day 2), separated by at least 1 week from the first one. The three groups were matched for age and gender distribution. On Day 1, participants trained a task of thumb-index opposition with their non-dominant hand, in order to increase their movement rate. The experimental groups differed in terms of the type of training performed. The main experimental group (12 subjects) executed the Motor Imagery and Electrical Stimulation (hereafter defined by ESMI) training session, during which they had to imagine a thumb-index opposition movement (kinaesthetic motor imagery) while receiving a synchronized electrical stimulus on their median nerve. Two different groups formed by 12 subjects each, executed a physical practice (hereafter PP) or a motor imagery (MI) training session, during which they had to execute or to imagine (kinaesthetic motor imagery) the thumb-index opposition movement. Noteworthy, during training all subjects were instructed to imagine or perform thumb-index opposition movements following a rhythmic acoustic cue. Acoustic cue was set at increasing frequencies ranging from 75% to 150% of individual maximal finger movements rate. Precisely, given the high rate of the acoustic cue, and the subsequent difficulty to imagine the complete movement, subjects were instructed to match the instant of contact between thumb and index to the acoustic cue. This procedure allowed us to synchronize the electrical stimulus to the imagined movements in ESMI training since electrical stimulus was delivered simultaneously to the acoustic cue (corresponding to the thumb-index contact phase of the imagined movement) (Figure 1).

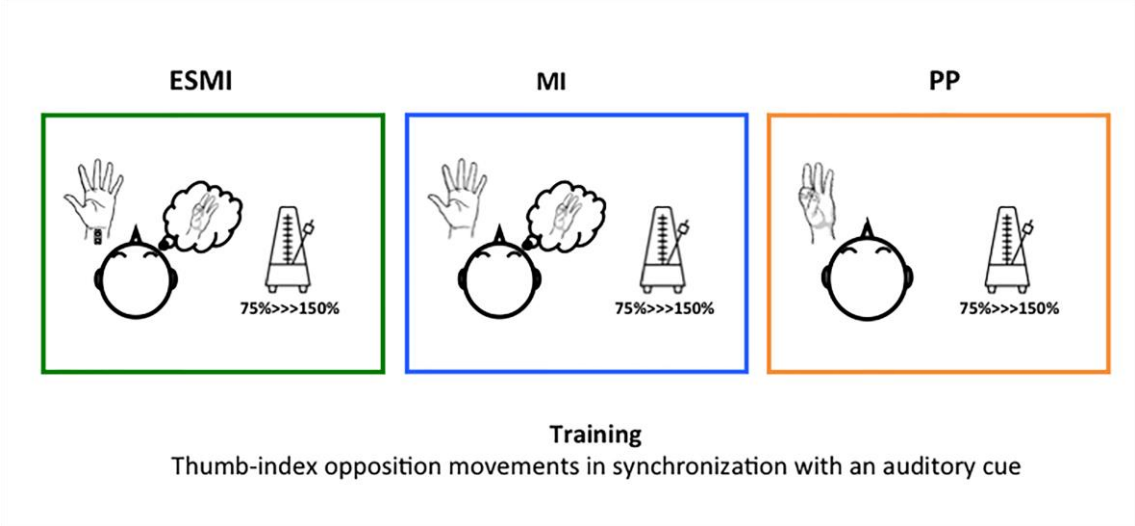

Figure 1. *Training sessions. During the Motor Imagery and Electrical Stimulation (ESMI) training session, subjects had to imagine a thumb-index opposition movement following a metronome while receiving a synchronized electrical stimulus on their median nerve. During physical practice (PP) or motor imagery (MI) training session, subjects had to execute or to imagine (kinaesthetic motor imagery) the thumb-index opposition movement following the acoustic cue. The acoustic cue was set at increasing frequencies (from 75% to 150% of individual maximal finger movements rate).*

Cortical excitability and thumb-index opposition performance at maximal speed was assessed before (Pre) and after (Post1) the training in each group. Further, immediately after the training sessions, the PAS25 protocol and the subsequent measurement of MEPs were applied to test for LTP-like plasticity occlusion in PMC. The following day in the retention session (Day 2), participants returned for performing thumb-index opposition task at maximal speed (Post2) (see Figure 2 for the experimental protocol).

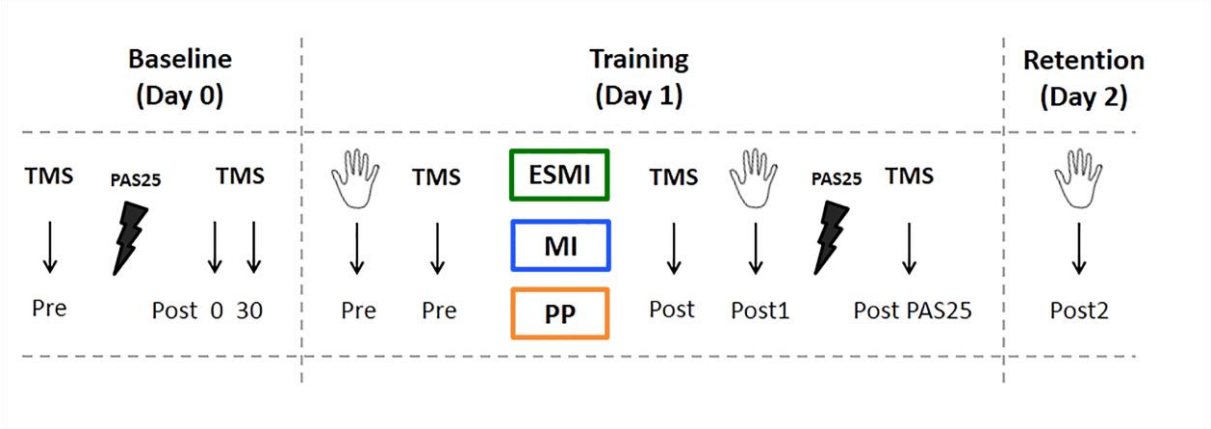

Figure 2. *Experimental protocol. On Day 0 we tested the effect of Paired Associative Stimulation (PAS25) protocol on motor evoked potentials (MEPs) by means of transcranial magnetic stimulation (TMS). On Day 1 participants were divided into three groups, performing different trainings. We tested the effect of different trainings on cortical excitability and on PAS25 induced-effects. Moreover, we tested the effect of training on finger movement's performance through an engineering glove, immediately after the training session and the following day (Day 2). MEPs, motor evoked potentials. ESMI, Electrical Stimulation and Motor Imagery; MI, Motor Imagery; PP, Physical Practice.*

Behavioral assessment

Subjects were seated on a chair wearing a sensor-engineered glove (Glove Analyzer System (GAS), ETT S.p.a., Italy) on their left hand[23]. We choose an eye-close paradigm to avoid confounding effects due to different kind of training (ESMI, MI, PP). To assess baseline performance, subjects had to execute a thumb-index opposition task at their maximal speed, two times, 30 seconds each, with 1 minute rest. We considered subjects average rate obtained from the two repetitions as individual 100% and we used this value to set the behavioral training. After each training session (Day 1) and one day later (Day 2), subjects repeated the thumb-index task two times 30 seconds each, in order to detect any speed change (Figure 2). We a priori choose to assess and train the non-dominant side because the behavioral task demanded a movement speed increase as outcome measure of training and

if the baseline frequency had been too fast (as for the dominant side in a very common task as thumb-index opposition movement), the amount of improvement would have been restricted from the physiological speed limit.

Behavioral training

From the individual 100% finger movements maximal rate we calculated three further percentages: 75, 125 and 150%. During training, subjects had to mentally perform (ESMI, MI) or execute (PP) the thumb-index opposition movements following the rhythm marked by a metronome, adjusted on the four percentages calculated before. The finger opposition task was executed (or imagined) for 20 seconds, two times for each percentage, from 75% to 150% of the individual maximal rate, for a total of 8 repetitions. Particularly, given the high rate of the acoustic cue subjects were instructed to match the instant of contact between thumb and index to the acoustic cue. Even though the aim of the training was that of raising the frequency of the thumb-index opposition movements, during the training sessions we initially set the metronome on 75% of subject's maximum speed, in order to allow subjects to familiarize with the acoustic cue and with imagined movement in the ESMI and MI groups. In ESMI training, participants had to kinaesthetically imagine the finger opposition task in rhythm with the metronome that, in turn, was synchronized with an electrical stimulus delivered over the left median nerve. Electrical stimulus was delivered simultaneously to the acoustic cue (corresponding to the thumb-index contact phase of the imagined movement. Electrical stimulation was delivered through a bipolar electrode over the left median nerve at the wrist (cathode proximal, constant square wave current, duration 200 microseconds, intensity set just above threshold for evoking a small twitch in the opponens pollicis muscle) (Digitimer D180 high voltage electric stimulator). In MI training participants were asked to kinesthetically imagine the same movement, without the peripheral stimulus, whereas in the

PP training they had to physically execute the task, always following the metronome during the training session (Figure 1).

Transcranial magnetic stimulation (TMS)

Single-pulses were delivered using a Magstim 200 stimulator (Magstim Co., Whitland, Wales, UK) with a monophasic current waveform connected to a figure-of-eight-shaped coil (external diameter of each loop, 9 cm) held tangentially to the scalp. The center of the junction of the coil was placed over the hand area of the right PMC at the optimal position (hot spot) to elicit MEPs in the non-dominant FDI, with the handle pointing backwards and $\sim 45^{\circ}$ away from the midline. The optimal coil location was searched by slightly moving the coil over the right PMC area until MEPs of maximal amplitude and lowest threshold in the left FDI were elicited. The exact coil position was marked by an inking pen. The stimulus intensity needed to evoke MEPs of approximately 0.8−1 mV peak-to-peak amplitude was defined (S1mV). This intensity was used to evaluate MEPs changes before and after training and PAS protocols (see below). Twenty MEPs were recorded at each testing time. The peak-to-peak MEP amplitude on single trials was used to calculate the mean MEP amplitude. On Day 0, MEPS were collected before PAS25 (PRE) after PAS25 (POST0) and 30 minutes after the application of PAS25 protocol (POST30). On Day 1 MEPS were collected before training (PRE training), after training (POST training) and after the application of PAS25 protocol (POST PAS25).

Paired associative Stimulation

The PAS25 protocol consisted of electrical stimuli of the left median nerve at the wrist level paired with single TMS pulses over the hotspot of the FDI muscle area of the right hemisphere, delivered 25ms later the electrical ones. Ninety paired stimulations were

applied at 0.05Hz over 30min, with an inter-stimulus interval (ISI) of 25ms. The TMS was delivered in the way described above, at S1mV stimulus intensity. The electrical stimulation was applied through a bipolar electrode (cathode proximal) using a square-wave pulse (duration, 200 microseconds) at an intensity of three times the perceptual threshold (Digitimer D180 high voltage electric stimulator). Participants were instructed to look in front of them at a black screen, so as to standardize the visual attentional load during the PAS protocols[24], and count the peripheral electrical stimuli they perceived. The MEPs evoked in the FDI were displayed online during the intervention to control for the correct coil position.

Electromyographic (EMG) recording

EMG was recorded through surface electrodes from the left FDI muscle using pairs of Ag-AgCl electrodes. Electromyographic signals (EMG) were digitalized, amplified and filtered (20 Hz to 1 kHz) with a 1902 isolated pre-amplifier controlled by the Power 1401 acquisition interface (Cambridge Electronic Design Limited, Cambridge, UK), and stored on a personal computer for display and later offline data analysis. Each recording epoch lasted 400 ms, of which 100 ms preceded the TMS. Participants were constantly reminded to always keep their hand relaxed during the whole experiment. EMG signal was monitored visually by the experimenter and trials with background EMG activity were excluded from analysis.

Power analysis

The main aim of the present study is to assess whether a training combining motor imagery and electrical stimulation is as effective in inducing plasticity in PMC as physical practice. We already showed that motor imagery training by itself induces changes in PMC plasticity (assessed as MEPs amplitude after the application of PAS25 protocol), that were minor than those induced by physical practice[18]. Therefore, we based our power analysis on the

differences in PMC plasticity induced by physical practice in our previous study using similar methodology[18]. In the previous study we adopted a within subject design with a sample size of n=9 participants who executed both physical practice and motor imagery training. This sample size yielded on 2-sided tests statistically significant within-group differences between MEPs amplitude after PAS25 protocol performed at rest or after physical practice session at a $p<0.04$ level, with corresponding power of about 92%. Here, the experimental design involved three different trainings (physical practice, motor imagery and motor imagery combined with electrical stimulation) and we also tested the retention of skill learning 24 hours later. Thus, to exclude learning effect due to repetition of training sessions, we adopted a between-subjects design. On the basis of all these assumptions, we anticipated that a sample size of 12 subjects per group would be adequate to show statistically significant differences within each group on neurophysiological parameters explored (MEPs amplitude after PAS25 protocol performed at rest on Day 0 vs MEPs amplitude after PAS25 protocol performed after a training session on Day 1) with a power of about 92%.

Data and statistical analysis

Data collected with the sensor-engineered glove were processed with a customized software. Finger opposition movements were described by: (i) movement rate, i.e. the number of contacts per second (Hz); (ii) touch duration (TD), i.e. the contact time between the thumb and index; (iii) inter-tapping interval (ITI), i.e. the time elapsing from end of contact between the thumb and index to the beginning of the subsequent contact. We considered the mean value between the two performances executed in the assessment phase before the training (Pre), immediately after the training (Post1) and the following day (Post2).

For movement rate, we also normalized data respect to baseline assessment (Pre) as follows:

Post1 = (movement rate Post1– movement rate Pre)/ movement rate Pre x 100)

Post2 = (movement rate Post2– movement rate Pre)/ movement rate Pre x 100)

Further, to measure also individual accuracy in increasing movement rate after training respect to the acoustic cue provided during training, we calculated, for each subject, the maximal rate provided by the acoustic cue and the performance rate after the training session and 24 hours later. Then, from these parameters we calculated the duration of the time interval set by the metronome (in ms, duration of the time interval between two successive acoustic cues, 1/Hz*1000) and the duration of the time interval reproduced (in ms, as processed by the customized GAS software as the sum of TD+ ITI). From these parameters we calculated a "temporal accuracy index" as the difference between the reproduced interval minus the set interval, to test if the rate at post-tests was close to the rate of the acoustic cue.

We checked that all variables were normally distributed (Shapiro-Wilk W test) and that sphericity was respected (Mauchly tests). Raw motor performance data (Movement rate, ITI and TD) were separately entered in a RM-ANOVA with time (Pre, Post1 and Post2) as within subjects factor and with group (ESMI, MI and PP) as between subjects factor. Further, normalized movement rate and temporal accuracy index data were entered in a RM-ANOVA with time (Post1 and Post2) as within subjects factor and with group (ESMI, MI and PP) as between subjects factor.

To evaluate the effect of PAS25 on cortical excitability on Day 0 (baseline session), mean MEPs amplitude was subjected to a RM ANOVA with time (PRE, POST0 and POST30) as within subject factor and GROUP (ESMI, MI and PP). To evaluate the effect of the different

trainings on PMC excitability and plasticity on Day 1, MEPs data were subjected to a RM ANOVA with time (PRE training, POST training and POST PAS25) as within subject factor and GROUP (ESMI, MI and PP) as between subjects factor. Further a Pearson correlation was performed between MEPs amplitude increase after PAS 25 on Day 0 (calculated as MEPs POST0- MEPs PRE/MEPs PRE*100) and MEPs amplitude increase on Day 1, after training session (calculated as MEPs POST training- MEPs PRE training/MEPs PRE training *100)

Statistical analysis was performed with SPSS 22.0. P-values of 0.05 were considered as threshold for statistical significance. *Post-hoc* analysis of significant interactions was performed by means of *t*-tests.

3.1.3 RESULTS

Preliminary findings

Data from the Day 0 evaluation (Figure 3), assessing the effect of the PAS25 protocol on subjects' plasticity, showed a significant effect of time ($F_{2,66}$=40.15; p<0.001). Post hoc analysis revealed an increase of MEPs amplitude after the PAS25 protocol (PRE vs POST0, p<0.001), maintained until 30 minutes after PAS25 application (PRE vs POST30, p<0.001). No significant effect of GROUP or GROUP*TIME interaction were found.

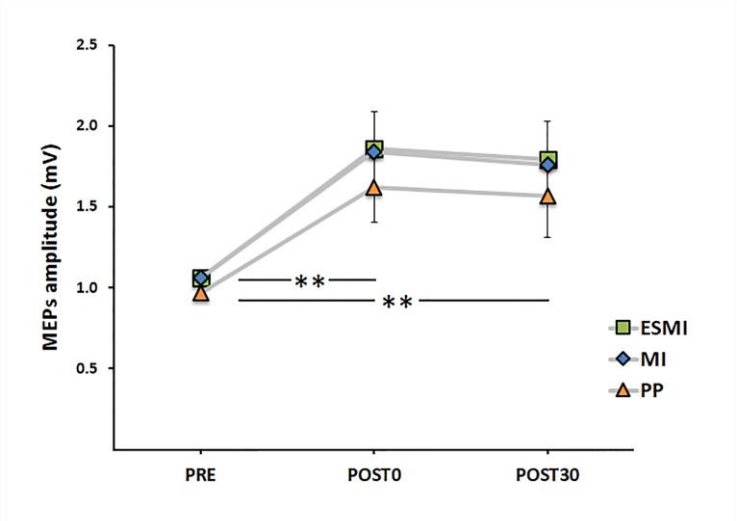

Figure 3. *PAS25 results on Day 0. Amplitude of the motor-evoked potentials (MEPs) recorded before (PRE), immediately after (POST0), and 30 min minutes (POST30) after the PAS25 protocol in the three groups. ESMI, Electrical Stimulation and Motor Imagery; MI, Motor Imagery; PP, Physical Practice.*

Motor performance

Participants were trained to physically or mentally (with or without peripheral nerve electrical stimulation) perform a task of thumb-index opposition, in order to increase their movement rate. We considered the mean movement rate value between the two performances executed in the assessment phase before the training (Pre), immediately after the training (Post1) and the following day (Post2).

Further we also normalized movement rate data collected after training and 24 hours later respect to baseline assessment. Finally, to measure individual accuracy in increasing movement rate after training with respect to the acoustic cue provided during training, we calculated, the difference between the time interval between two successive acoustic cues provided by the metronome set at 150% of maximal voluntary rate and the time interval between two successive movements reproduced by the subjects immediately after the training and 24 hours later (temporal accuracy index).

Statistical analysis on raw movement rate data showed a significant time *group interaction ($F_{4,66}$=2.98; p=0.025). Post hoc analysis revealed that movement rate increased after training in all the experimental group (Pre vs Post1, p always < 0.001), but whereas this increase was maintained 24 hours later in ESMI (Pre vs Post2, p <0.001) and PP groups (Pre vs Post2, p = 0.001), in the MI group movement rate returned comparable to baseline values (Pre vs Post2, p = 0.36) (Figure 4A).

Further, when we compared normalized movement rate data, a significant effect of group ($F_{2,33}$=3.10; p=0.045) was found. Post hoc analysis showed that movement rate changes in

Day 1 and Day 2 were similar in the ESMI and in physical practice conditions (ESMI vs PP, p=0.70) whereas the increase in movement rate after training on Day 1 and the retention of the acquired skill on Day 2 were smaller in the MI group respect to the other two (MI vs ESMI, p=0.029; MI vs PP, p=0.040) groups (Figure 4B). This finding was also confirmed by statistical analysis on individual accuracy respect to the acoustic cue provided during the training. Indeed, RM-ANOVA showed a significant effect of group ($F_{(2,33)}$=4.83, p=0.014). ESMI and PP group were more accurate, showing a smaller temporal accuracy index on Day 1 (ESMI, 41.78 ± 9.73 ms; PP, 45.94 ± 11.06 ms) and on Day 2 (ESMI, 54.01 ± 12.74 ms; PP, 58.14 ± 12.61 ms) respect to MI (Day 1, 50.02 ± 11.57 ms; Day 2, 68.37 ± 8.08 ms) (ESMI vs MI, p=0.004; PP vs MI, p=0.04).

When we analysed the kinematic parameters of finger movements, statistical analysis showed that the touch duration (TD, i.e., the time spent in the contact between the thumb and the index) decreased with training in all groups (time, $F_{2,66}$=9.09; p<0.001) immediately after training (Pre vs Post1, p<0.001) and returned to baseline values 24 hours later (Pre vs Post2, p=0.053) (Figure 4C).

Differently, related to inter-tapping interval (ITI), i.e. the time elapsing from end of contact between the thumb and the index and the beginning of the subsequent contact, statistical analysis showed a significant group*time interaction ($F_{4,66}$=2.90; p=0.028). Post hoc analysis revealed that in ESMI and PP groups ITI decreased immediately after training (ESMI, Pre vs Post1, p=0.002; PP, Pre vs Post1, p<0.001) and 24 hours later (ESMI, Pre vs Post2, p<0.001; PP, Pre vs Post2, p<0.001) (Figure 4D). Differently, in MI group ITI decreased immediately after training (Pre vs Post1, p=0.018), but returned to baseline values 24 hours later (Pre vs Post2, p=0.72) (Figure 4D).

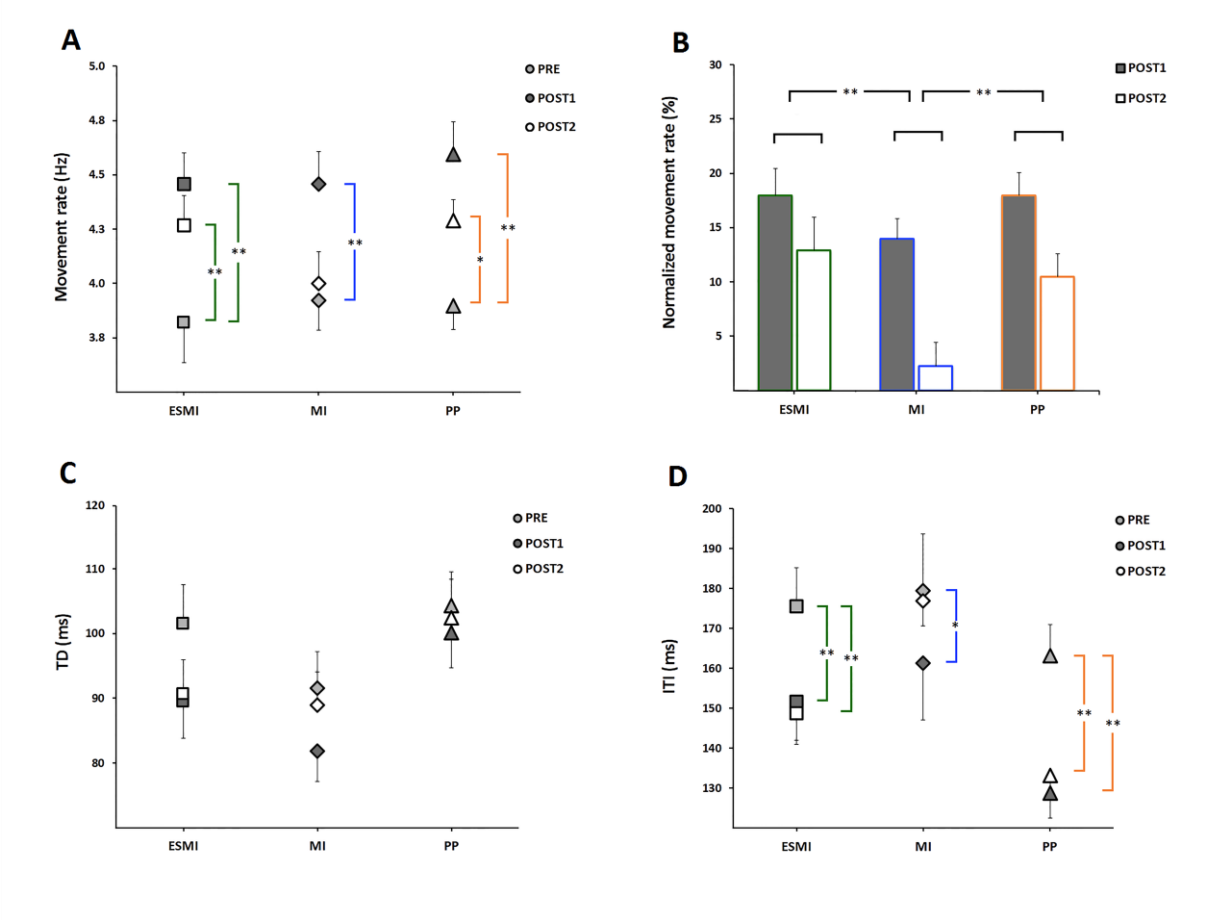

Figure 4. *Motor performance. The effect of the different trainings on movement rate (A-B), touch duration (TD, panel C) and inter-tapping interval (ITI, panel D) is reported. In panel A-C-D, squares represent subjects of ESMI, diamonds represent MI subject and triangles correspond to PP subjects. In panel B the % change in movement rate is reported on Post1 (calculated as (Post1– Pre)/ Pre x100) and on Post2 testing times (calculated as (Post2 – Pre)/ Pre x100). Vertical bars indicate standard error of the mean (SEM). Asterisks indicate the level of significance (* p< 0.05; ** p<0.01).*

<u>Effect of different trainings on PAS-induced effects</u>

Regarding cortical excitability data, RM-ANOVA showed a significant effect of time ($F_{2,66}$= 35.83, p<0.001), with a significant group x time interaction ($F_{4,66}$=3.98, p=0.006). Post hoc analysis revealed that MEPs amplitude increased immediately after training after ESMI training (ESMI: PRE training vs POST training, p=0.001) as well as after physical practice (PP: PRE training vs POST training, p<0.001), and motor imagery training (MI: PRE training vs

POST training, p<0.001) (Figure 5). No differences between groups were found regarding MEPs amplitude on the POST training testing time (ESMI POST training vs MI POST training, p=0.69; ESMI POST training vs PP POST training, p=0.48; MI POST training, vs PP POST training, p=0.75). Further, we found a significant correlation between MEPs amplitude increase after PAS25 on Day 0 and MEPs amplitude increase on Day 1, after training session in all groups (ESMI, r=0.64, p= 0.026; PP, r=0.65 p=0.021 MI, r=0.72 p= 0.007), indicating that in all groups "better" PAS-responders showed a stronger increase in MEPs amplitude immediately after training.

After the PAS25 protocol, groups that performed ESMI and PP training showed a significant decrease in MEPs amplitude (ESMI: POST training vs POST PAS25 p=0.001; PP: POST training vs POST PAS25 p<0.001), without differences between the two training protocols (POST PAS25: ESMI vs PP p=0.41), whereas when PAS25 was applied after motor imagery training MEPs amplitude did not change (MI: POST training vs POST PAS25 p=0.17) (Figure 5). After the administration of the PAS25 protocol (POST PAS25 testing time) MEPs amplitude in the MI group was significantly larger than MEPs amplitude in the ESMI group (POST PAS25: MI vs ESMI, p=0.045) and in the PP group (POST PAS25: MI vs PP, p=0.013).

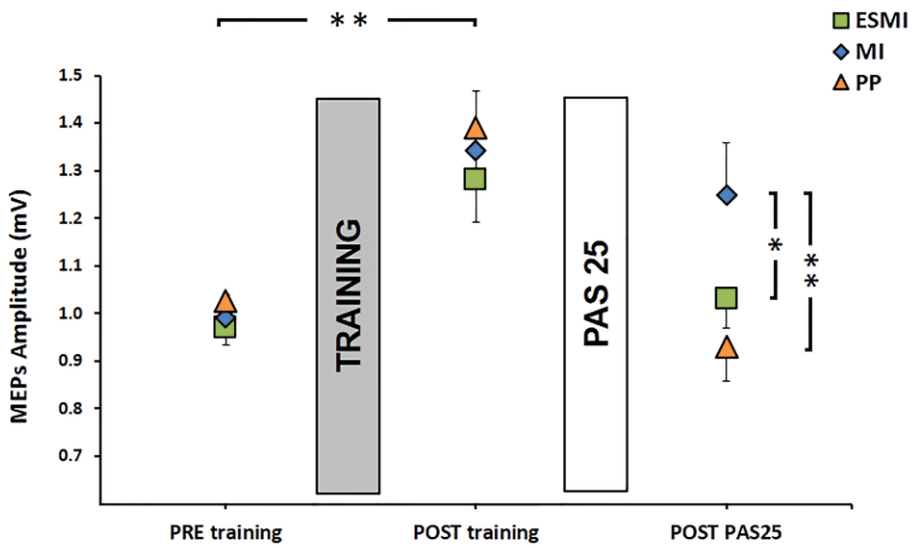

Figure 5. *Effect of training on PAS25 in the three groups. Cortical excitability data of the three groups (ESMI, MI, PP) are shown. Squares represent subjects of ESMI, diamonds represent MI subject and triangles correspond to PP subjects. MEPs amplitude, in mV, is depicted before and after each training session (dark grey bars) and after PAS25 plasticity protocol (light grey bars). Vertical bars indicate standard error of the mean (SEM). Asterisks indicate the level of significance (* p< 0.05; ** p<0.01).*

3.1.4 DISCUSSION

The main findings of this study include the following: (1) training through physical practice (PP) and motor imagery combined with peripheral nerve stimulation (ESMI) similarly induced motor learning, more than training through motor imagery alone (MI); (2) the three types of training (ESMI, MI or PP) induced an increase in cortical excitability; (3) all the training sessions (ESMI, MI or PP) prevented the subsequent PAS25-induced LTP-like plasticity, but the occlusion of LTP-like plasticity was stronger after both the ESMI and physical practice training sessions than after motor imagery alone; (4) this neurophysiological finding was

paralleled by better retention of the newly acquired skill, reflected by performance in the second day of practice, in the ESMI and PP groups with respect to MI group.

Our behavioural data showed that after training, motor performance improved in all groups, even if improvements observed after ESMI and PP trainings were stronger than after MI training, in accordance with previous studies[18,25,26]. Further, retention of the acquired skill, tested as motor performance on the subsequent day, was present in ESMI and PP groups but not in MI group. Particularly, the decrease in the 'inter tapping interval', that can be interpreted as a pure 'motor time' during finger opposition movements, was maintained until 24 hours after training in the ESMI and PP groups.

As already discussed in a previous study[18], differences in motor learning between motor imagery and physical practice may be explained by different sensorimotor mechanism used during training. While physical practice involves both motor and sensory processes in order to improve the quality and the efficiency of the movement, training through imagination rests on internal forward models, which predict the future state without any bottom-up feedback. Because of this difference, practice is probably less accurate in MI, leading to a smaller improvement in motor performance[18,25,26]. Recent evidence in the literature showed that the combination of MI and peripheral nerve electrical stimulation above motor threshold was able to influence PMC excitability similarly to voluntary movement[19,20]. However no behavioural data are available in the literature so far on the efficacy of combined MI and peripheral stimulation training. Our behavioural findings show that when participants received a sensory feedback combined with the movement imagination during training, they improved their behavioural performance to the same extent as participants who executed the physical practice training.

Somatosensory feedback is able to redefine many aspects of a motor pattern, such as movement accuracy, pattern frequency and force adjustments for on-going movements[27–29]. Accordingly, a reduction of somatosensory inputs by short-term immobilization of a limb impairs motor performance, even if this modification quickly decreases during trial-by-trial movement repetition[30]. Short-term limb immobilization also affects cortical excitability and plasticity of the motor cortex contralateral to the restricted limb[31–35], and these modifications are strongly dependant from the deprivation of sensory inputs. Indeed, these cortical effects were partially counteracted when somatosensory inputs were delivered to the restricted limb during the immobilization period[32], supporting the general idea that sensory inputs crucially shape somatosensory networks. Accordingly, the provision of motor imagery by itself was not able to cope with the corticomotor depression induced by immobilization[36].

Here, regarding PMC excitability, we observed an increase in MEPs amplitude after all the different training sessions. We decided a priori not to include a training with peripheral stimulus alone since it has already been showed that it doesn't lead to any modification of PMC neuroplasticity[37], unless when provided at higher frequencies or for longer stimulation periods[38–40]. The increase of PMC excitability induced by the "augmented" MI training was similar to that observed after physical practice. In contrast with our previous study[18] and others[39] the increase of PMC excitability after motor imagery training was similar to that observed after physical practice. However we think that a possible explanation of this finding could deal with the difference between the tasks adopted for the motor imagery training in these studies. Indeed, at difference with our previous study, subjects were asked to imagine thumb-index opposition movements following a rhythmic external auditory cue. Auditory cues have already been used to improve motor control and motor learning and the

association of an external auditory cue with physical practice has been proven to promote motor skill acquisition and to get sport performance more efficient[41–43]. Moreover, the use of auditory cues significantly influences motor imagery increasing subjects' imagery vividness, likely by triggering a separate neural system, the cerebello-thalamo-cortical, preferentially used in movement based upon external sensory cues[44]. Here we also showed that "better" PAS25 responders in the three groups were those subjects who showed a larger increase in PMC excitability immediately after the training session. These data confirm data in the literature suggesting that PAS25 and motor training by physical practice are likely to induce LTP-like plasticity on the same neural population in PMC[45–48] , and enlarge this concept to different types of training like motor imagery training and training combining motor imagery and electrical stimulation.

Concerning the PAS25 effect on PMC excitability after training, in accordance with strong evidence in the literature, we found that physical practice prevented the subsequent PAS25-induced LTP-like plasticity in PMC[45,46]. Several studies showed that in humans, learning and non-invasive brain stimulation protocols, which individually are able to induce LTP-like plasticity in PMC, interact with each other. Indeed, for the link between synaptic plasticity and memory formation to be confirmed, the principle of occlusion is required[49]; in other words, saturation of synaptic plasticity in a network should occlude new memory encoding[49]. This principle has been demonstrated for motor learning when interacting with non-invasive brain stimulation protocols able to evoke long-lasting changes of PMC excitability, as for instance paired associative stimulation[45,46,48,50]. We have however to mention that, even if there is robust evidence in supporting the interaction between PAS25 induced-plasticity and motor learning, the mechanisms of action of paired associative stimulation protocols are not completely clear, possibly involving cortical plasticity in other cortical areas and

interconnected networks apart PMC[51] and not always following temporal "spike timing-dependent plasticity" rules, likely depending also on the specific neuronal populations stimulated and on the activity state of the cortex[52,53].

However, here we showed for the first time that a training based on the combination of MI and a somatosensory afferent stimulation was able to induce the same effect as physical practice did, preventing the subsequent PAS25-induced LTP-like plasticity, in a stronger manner than MI alone. Our findings are in accordance and expand those by Mrachacz-Kersting and coworkers (2012). These authors showed that concomitance between motor imagery and the ascending volley due to the peripheral nerve stimulation could lead to a significant increase in cortical excitability[39]. Our neurophysiological finding was paralleled by better retention of the newly acquired skill, as reflected by performance in the second day of practice, in ESMI and PP groups respect to MI group. Particularly, when we analysed the kinematic properties of finger opposition movements, we found that changes in the time dedicated to movement (inter-tapping interval, ITI) persisted until 24 hours after training in ESMI and PP groups, while changes in the time dedicated to the contact between thumb and index (touch duration, TD) were similarly observed in the three groups only immediately after training. Although ITI is likely to represent a pure motor phase, TD may be regarded as the combination of a sensory phase and a motor preparation phase in which the subsequent movement is correctly planned prior to execution. The selective decrease of ITI maintained until 24 hours after the training session in ESMI and PP groups may suggest that these practice sessions favoured the formation of a new motor memory. For physical practice it has already been demonstrated that the magnitude of occlusion of LTP-like plasticity after training (that is an index of the amount of LTP-like plasticity used during motor learning), was associated with better performance and more resilience to retrograde interference from

a second task on a subsequent day, suggesting a retention mechanism[7]. Here we showed that similar mechanisms operated when learning is acquired through motor imagery training and can be enhanced through the provision of somatosensory inputs during motor imagery.

Further, our results fit in a novel scenario supporting that the combination of somatosensory inputs (provided by peripheral nerve stimulation) with cognitive processes of movement (motor imagery or action observation) could lead to strong changes in cortical excitability and motor performance. Indeed, when action observation was delivered in conjunction with a peripheral nerve stimulation, it induced an increase of the PMC excitability which outlasted the stimulation period, induced learning of a newly trained skill and prevented the subsequent induction of LTP-like plasticity[37,54,55]. Taken together this piece of evidence supports the use of protocols combining cognitive representation of movement and somatosensory inputs in order to induce LTP-like plasticity in PMC and learning and consolidation of a new motor skill.

Some issues still remain open and deserve to be explored in future studies. First, we a priori chose to test LTP-like plasticity with PAS25 protocol that is dependent on sensory afferent stimulation.

However, to better elucidate different mechanisms underlying LTP-like plasticity in PMC induced by motor imagery training (with or without electrical stimulation) it will be interesting to use different LTP-like plasticity induction protocols as theta burst stimulation or repetitive TMS. Second, on Day 2, we tested only for retention of motor skill learning, but in future studies it will be of interesting to assess also for long lasting changes in PMC excitability induced by different types of training.

In conclusion, our findings emphasize the role played by somatosensory inputs during motor imagery training. It has been suggested that brain activation during motor imagery likely corresponds to activation of the neural representations of a ‘‘potential’’ movement that is retrieved volitionally from motoric memory[17]. Here we showed that during motor imagery sensory feedback might be crucial in inducing LTP-like plasticity in PMC. In other words, when combined with sensory stimulation, the cognitive retrieval of motor plan is able to induce plasticity in PMC (changes in synaptic efficiency) that in turns corresponds to motor learning and consolidation of a new motor memory. These results suggest combining motor imagery and somatosensory stimulation to induce motor learning, as in a rehabilitative setting or in sport.

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3.2 TO MOVE OR NOT TO MOVE: SELECTIVE SENSORIMOTOR MODULATION OPERATES DURING COGNITIVE REPRESENTATION OF MOVEMENT

3.2.1 INTRODUCTION

Motor imagery (MI) is viewed as a window to cognitive motor processes and particularly to motor control. MI consists in the mental simulation of a movement without the completion of the actual movement[1] and is a well-established alternative way for motor learning[2–4]. Indeed, a mental training can improve motor execution in terms of strength, speed and accuracy[5–8].

Similar activations between movement execution (ME) and MI have been found in the sensorimotor cortex, the cerebellum and the basal ganglia[3,9–11]. However, even if primary motor cortex (PMC) is a key area shared between MI and ME, PMC activation is stronger during ME than MI[12,13], likely because in MI the final command to execute the movement is not sent or movement is prevented. Interestingly, this mechanism operates since the planning phase of mental movements, prior to task execution. Before the expected movement onset, imagining to move causes an increase in corticospinal excitability and a decrease of intracortical inhibition that follows the temporal profile, but is to a much lesser extent than real performance[14].

During the planning phase of executed movements, but just before the movement onset, PMC activity is selectively modulated by somatic sensory inputs, in relation to the task goal[15,16]. Sensorimotor modulation can be tested by means of a transcranial magnetic

stimulation (TMS) technique, namely short afferent inhibition (SAI). SAI involves pairing an afferent nerve stimulation with TMS of the PMC[17,18]. When TMS stimulus is preceded 20 ms earlier by peripheral nerve stimulation, excitability of the PMC is reduced[17]. During movement execution, SAI magnitude is selectively reduced in the muscle involved in the task, at movement initiation (after a 'go' cue), likely to focus the neural activity in PMC[16]. To date we are not aware if a similar mechanism operates also during the planning phase of imagined movements. Such information could shed light on the role of the afferent somatic inputs during motor imagery, that is still open to debate. Some authors recognise MI as a cognitive motor process[19], in which information about the motor act has to be processed from long-term to working memory[20]. However, it has also been showed that MI is influenced by postural manipulations and biomechanical constraints, suggesting a role of kinaesthetic afference in the embodied properties of MI[21,22]. Speaking more generally, such information will give insight into the sensorimotor mechanisms operating during the cognitive representation of movement.

Here, in a first experiment, we assessed sensorimotor modulation at movement initiation of mental movements. We adopted the paradigm described by Asmussen and colleagues[16], testing SAI at different times before the onset of executed or mental movement and from a muscle involved and one uninvolved in the task. If online afferent feedback contributes to motor imagery, then a modulation of SAI at movement initiation should occur, in a very specific way as it happens during movement execution; i.e., decreased SAI only in the muscle involved in the task. If this is the case, we also hypothesized that SAI modulation at movement initiation will be of lower magnitude during MI with respect to ME depending, at least partially, on the level of PMC activity. To test for this, in a second experiment, we artificially modulated the amount of PMC activity, by increasing it through neuromodulation,

and we analysed if such an intervention increased sensorimotor modulation prior to mental or executed movement.

3.2.2 MATERIALS AND METHODS

Subjects

Fifteen right-handed subjects were recruited for the first experiment (mean age 23.18 ±2.23, 8 males) and 20 right-handed subject (mean age 22.35±1.95, 6 males) for the second experiment. All subjects were in good health, without any nervous, muscular, orthopaedic or cognitive disorders. Right arm dominance was determined by means of the Edinburgh Handedness inventory[23]. In order to assess subject's imagination vividness, we administered the "Kinaesthetic and Visual Imagery Questionnaire" (KVIQ-10). The KVIQ assesses the clarity of the image (visual: V subscale) and the intensity of the sensations (kinesthetic: K subscale) that the subjects are able to mentally create from the first-person perspective. Rating consists of a five-point ordinal scale, where the higher is the score the higher the intensity of the sensations associated with the imagined movement or the clarity of the visual image. The experimental protocol was approved by the ethics committee of the University of Genoa and was carried out in agreement with legal requirements and international norms (Declaration of Helsinki, 1964).

Experiment 1

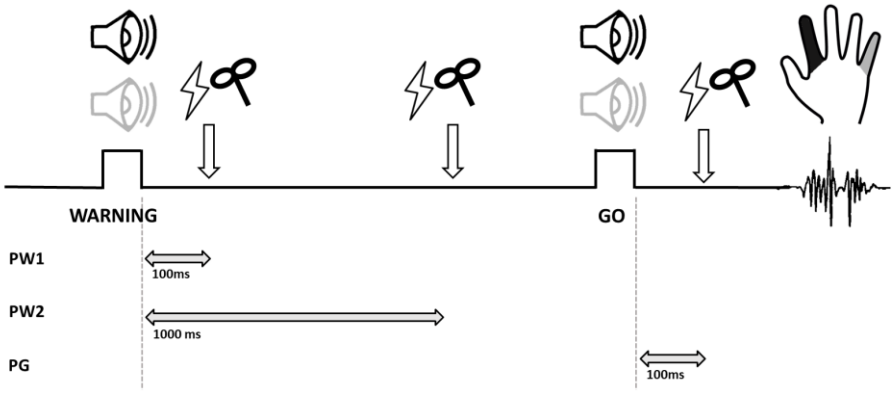
Subjects were seated on a comfortable chair responding to a choice reaction time task with a finger movement as a response to an acoustic cue. We chose an eye-closed paradigm to avoid confounding effects due to different kinds of movement (MI or ME). Each movement

was associated with a sound: a high tone for the 2nd finger (index) abduction and a low tone for the 5th finger (little finger) abduction. Sound cues were produced with a customizable microcontroller board (Arduino Uno). Every trial was composed of two acoustic sounds: a 'warning' cue, that allowed participants to understand what finger they had to move, and a 'go' cue, after which they had to move or imagine abducting the selected finger. We tested short afferent inhibition (SAI) only from the first dorsal interosseus muscle (FDI), involved only in the 2nd finger abduction, at different testing times, while subjects moved or imagined moving the 2nd or the 5th finger abduction. In this way we recorded SAI from a muscle involved in the task (in the case of 2nd finger abduction) and from a muscle uninvolved in the task (in the case of 5nd finger abduction). SAI was evaluated 100 ms (Post Warning 1; PW1) and 1000 ms (Post Warning 2, PW2) after the 'warning' cue, and 100 ms after the 'go' cue (Post Go, PG) (Figure 1A). Trials in which EMG activity before the 'go' cue was larger than during rest were rejected. The experimental session was divided into two randomized parts, in the first one participants had to execute the movement (ME), in the second one they had to kinaesthetically imagine fingers abduction (MI).

Experiment 2

Following the results of Experiment 1, in Experiment 2 we evaluated SAI in the preparation phase of movement execution and motor imagery 100 ms after the 'go' cue (PG). Subjects came to the laboratory on two different days to participate to Experiment 2. Sensorimotor modulation assessment during MI and ME tasks was done after anodal excitatory transcranial direct current stimulation (tDCS) or sham ineffective stimulation in two different days. The order of MI and ME task was randomized between subjects. The order of anodal or sham stimulation sessions was also randomized (Figure 1B).

A



B

Figure 1. *Experimental paradigm.* ***(A)*** *Experiment 1. Sound icons represent 'warning' and 'go' cues. The light grey audio symbol represents low tone (5th finger abduction) the dark grey one stands for high tone (2nd finger abduction). Vertical arrows indicate short afferent inhibition (SAI) from first dorsal interosseus muscle (FDI) testing times. SAI was tested at post "warning cue" (PW1), post "warning cue" 2 (PW2) and post "go cue" (PG) testing times.* ***(B)*** *Experiment 2. The head with coloured squares indicates transcranial direct current (tDCS) stimulation. SAI was tested only at post "go cue" (PG) testing time after anodal tDCS or sham stimulation.*

Electromyographic (EMG) recording

EMG was recorded with silver disc surface electrodes placed on a tendon belly arrangement over the bulk of the right first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles. Electromyography signals were amplified and filtered (20Hz to 1 kHz) with a D360 amplifier (Digitimer). The signals were sampled at 5000 Hz, digitized with a laboratory interface (Power 1401, Cambridge Electronic Design, Cambridge, UK), and stored on a personal computer for display and later offline data analysis.

Transcranial magnetic stimulation (TMS)

Single-pulses were delivered using a Magstim 200 stimulator (Magstim, UK) with a monophasic current waveform connected to a figure-of-eight-shaped coil (external diameter of each loop, 9 cm) held tangentially to the scalp. The center of the junction of the coil was placed over the hand area of the controlateral PMC at the optimal position (hot spot) to elicit Motor evoked potentials (MEPs) in the right FDI, with the handle pointing backwards and ∼45◦ away from the midline. With this coil orientation, the induced current flowed in an anterior–medial direction approximately perpendicular to the central sulcus. The optimal coil location was searched by slightly moving the coil over the right PMC area until MEPs of maximal amplitude and lowest threshold in the left FDI were elicited. The exact coil position was marked by an inking pen to ensure an accurate positioning of the coil throughout the experiment. At the beginning of each experiment, the stimulus intensity needed to evoke MEPs of approximately 0.8–1.0 mV peak-to-peak amplitude was defined (S1mV).

Short afferent inhibition (SAI) protocol

SAI was tested with a suprathreshold test TMS stimulus over the PMC representation of right FDI, adjusted to produce MEPs of 1 mV in amplitude (S1mV), preceded (20ms inter-stimulus interval) by an electrical conditioning stimulus over the contralateral median nerve at the right wrist. Electrical stimulation (ES) was delivered through a bipolar electrode (cathode proximal) using a square-wave pulse (duration, 200 microseconds (200 μs) an intensity set just above threshold for evoking a small twitch in the opponens pollicis muscle (Digitimer D180 high voltage electric stimulator).

Ten conditioned trials at 20 ms inter-stimulus interval (ES+ TMS) and 10 unconditioned trials (TMS, TEST) were recorded before the real and the imagined movement at PW1, PW2 and PG testing times, for each to-be-moved finger (2^{nd} or 5^{th}) (60 conditioned trials and 60 unconditioned trials for both the MI and ME tasks). SAI was also assessed at rest, with 20 conditioned trials at 20 ms inter-stimulus interval and with 20 unconditioned (TEST) stimuli delivered randomly during the experiment.

We also define hereafter “homotopic stimulation” when SAI was assessed from right FDI during 2^{nd} finger abduction (MI or ME); “heterotopic stimulation” when SAI was assessed from right FDI during 5^{th} finger abduction (MI or ME).

Notably, for Experiment 2, when necessary, the intensity of the TMS stimulus for SAI assessment, was adjusted, after the neuromodulation session to produce MEPs of 1 mV in amplitude (PRE stimulation mean TMS intensity, 40.85 ± 5.4; POST stimulation mean TMS intensity, 39.23 ± 5.8). For Experiment 2, SAI was tested at rest and at PG only testing time.

The triggers for electrical stimulation and TMS were generated by the Power 1401 (Cambridge Electronic Design, CED, Cambridge, UK) and temporally synchronized with the auditory signals thanks to CED Signal Software.

Transcranial direct current stimulation (tDCS)

A direct current stimulator (BrainSTIM, E.M.S. s.r.l.) delivered a constant current of 1.5 mA, through two sponge electrodes (surface 25 cm^2) in saline- soaked solution. To increase cortical excitability of PMC in the active stimulation condition the anode electrode was placed over the left PMC, located using C3 in accordance with the international 10–20 system of measurement, while the cathode was placed over the contralateral supraorbital area (REAL stimulation, andodal tDCS, a-tDCS)[24]. The stimulation session lasted 20 minutes. In the SHAM stimulation condition, electrodes were placed similarly to the active condition, the current was ramped-up for 20 s until it reached 1.5 mA, then ramped-down in 20 s and turned off without participant's knowledge, so that the participant felt the same sensation of active stimulation. This sham condition has been confirmed to produce no effects on brain excitability. The order of the stimulation conditions was randomized and counterbalanced across subjects.

Data and statistical analysis

For TMS, the peak-to-peak MEPs amplitude on single trials was used to calculate the mean MEPs amplitude in each block, without peripheral stimulation (TEST).

SAI at rest was calculated as a ratio of the conditioned MEP on the unconditioned one (SAI = $\frac{MEP\ conditioned}{MEP\ unconditioned}$). Moreover, SAI value at each testing time (PW1, PW2 and PG) was normalized to SAI at rest (SAI $_{ratio}= \frac{SAI\ testing}{SAI\ at\ rest}$). This normalization shows data as an increase

or a decrease of SAI. If the value is >1 it indicates a SAI reduction (increased MEPs) and, on the contrary, if the value is <1 there is a SAI increase (decreased MEPs)[16].

Before performing further statistical analysis, we checked that all variables were normally distributed (Shapiro-Wilk test) and that sphericity was respected (Mauchly tests).

Experiment 1

First, we compared SAI at rest between the tasks (ME and MI) with a Repeated Measure ANOVA (RM-ANOVA) with TASK (ME and MI) and CONDITION (Unconditioned MEPS and Conditioned MEPs).

To evaluate the effect of movement preparation on SAI modulation, SAI_{ratio} was subjected to a three-way Repeated Measure ANOVA (RM-ANOVA) with TASK (ME and MI), TESTING TIME (PW1, PW2 and PG) and MOVING FINGER (2^{nd} finger, 5^{th} finger) as within subject factors. Furthermore, to investigate a possible relationship between SAI modulation during ME and MI, a correlation between SAI Ratio at PG testing time during MI and ME was analysed with Pearson correlation coefficient. Finally, we correlated SAI modulation in PG block during motor imagery and the ability to kinaesthetically imagine, using KVIQ-10 scoring (item #K3, #K5, #K6, #K8, #K9). For this analysis we used Spearman's rho correlation coefficient, as KVIQ #K values where not normally distributed (Shapiro-Wilk test, p always < 0.05).

Experiment 2

To assess whether the application of a-tDCS induced changes in SAI, SAI at rest (SAI = $\frac{MEP\ conditioned}{MEP\ unconditioned}$) recorded after REAL and SHAM stimulation were compared by means of a paired t-test. Then, to evaluate a-tDCS effects on SAI modulation, SAI_{ratio} was subjected to a RM-ANOVA with TASK (ME, MI), MOVING FINGER (2^{nd} finger, 5^{th} finger) and STIMULATION (REAL, SHAM) as within subject factors.

Post-hoc analysis of significant interactions was performed by means of *t*-tests applying the Bonferroni correction for multiple comparisons when necessary. Statistical analysis was performed with SPSS 22.0. P-values of 0.05 were considered as threshold for statistical significance.

3.2.3 RESULTS

Participants were trained to physically (ME) or mentally (MI) perform a reaction time task of finger abduction, in response to an acoustic cue. If the 'warning' acoustic cue was a high tone, the required movement, after the 'go' cue was an index finger abduction (2^{nd} finger), if it was a low tone the movement was a little finger abduction (5^{th} finger). We tested SAI at two testing times between the cues (PW1, PW2) and at one after the 'go' cue and before EMG activity (PG), only from right FDI muscle involved in 2^{nd} finger abduction, but not in 5^{th} finger abduction.

In all subjects during both the ME and MI tasks, when TMS was preceded by ES, MEPs amplitude significantly decreased respect to TMS alone (Table 1). Accordingly, the statistical analysis showed a significant effect of CONDITION ($F_{1,14}$=7.97, p<0.001), but no significant effect of TASK or interaction TASK*CONDITION.

Table 1. Unconditioned (TMS) and conditioned (ES+TMS) MEPs amplitude at rest. Mean MEPs amplitude (mV) followed by standard error is presented.

TASK	Unconditioned MEPs (TEST)	Conditioned MEPs
Movement execution	1.04 ± 0.05	0.29 ± 0.10
Motor imagery	1.01 ± 0.04	0.31± 0.12

Experiment 1: Sensorimotor integration during MI and ME preparation and initiation

Statistical analysis showed that sensorimotor modulation occurred in the cortical representation of the moving finger during the planning phase of either an executed movement and an imagined one, though with differences between executed and imagined movement in terms of strength of sensorimotor modulation (Figure 2). According to previous finding by Asmussen and co-workers, RM-ANOVA showed significant effects of MOVING FINGER ($F_{1,14}$=18.45, p=0.007) and TESTING TIME ($F_{2,28}$=16.92, p<0.001) and a significant TESTING TIME x MOVING FINGER interaction ($F_{2,28}$=18.85, p<0.001). Post hoc analysis displayed that (i) SAI was modified only if the to-be-moved finger was the same to be tested (2nd finger) (p= 0.001); and that (ii) inter-cues evaluated moments (PW1 and PW2) differed from the after 'go' cue moment (PW1 vs PG, p<0.001; PW2 vs PG, p=0.001) without differences between the two (PW1 vs PW2, p=0.276). Finally, post hoc analysis of the TESTING TIME x MOVING FINGER interaction displayed that in the case of homotopic stimulation, SAI changed only during PG (for 2nd finger abduction: PW1 vs PW2, p=0.589, PG vs PW1or PW2, p always < 0.001), whereas there were no differences between the different

testing times in the case of heterotopic stimulation (for 5th finger abduction, p always > 0.05).

Related to our experimental question we found a significant effect of the TASK x TESTING TIME interaction ($F_{2,28}$=4.77; p=0.016). Post hoc analysis showed that SAI recorded at the "after go cue" testing time (PG) was significantly reduced respect to SAI recorded at the inter-cues testing times (PW1 and PW2) both when the task was to execute the movement (ME: PG vs PW1, p=0.001; PG vs PW2, p=0.001) and when the task was to imagine the movement (MI: PG vs PW1, p=0.001; PG vs PW2, p=0.007). Noteworthy, post hoc analysis of the TASK x TESTING TIME interaction showed also that SAI modulation recorded at the "after go cue" testing time (PG) was stronger when the task was to execute the movement than when the task was to imagine the movement (ME vs MI p<0.001). This latter finding was confirmed by a general main effect of CONDITION ($F_{1,14}$=16.51; p=0.001) displaying that SAI modulation was larger during movement execution than during motor imagery. Finally, we did not find a significant TASK x TESTING TIME x MOVING FINGER interaction ($F_{2,28}$=0.75; p=0.48), showing that SAI modulation followed the same pattern in MI as in ME condition.

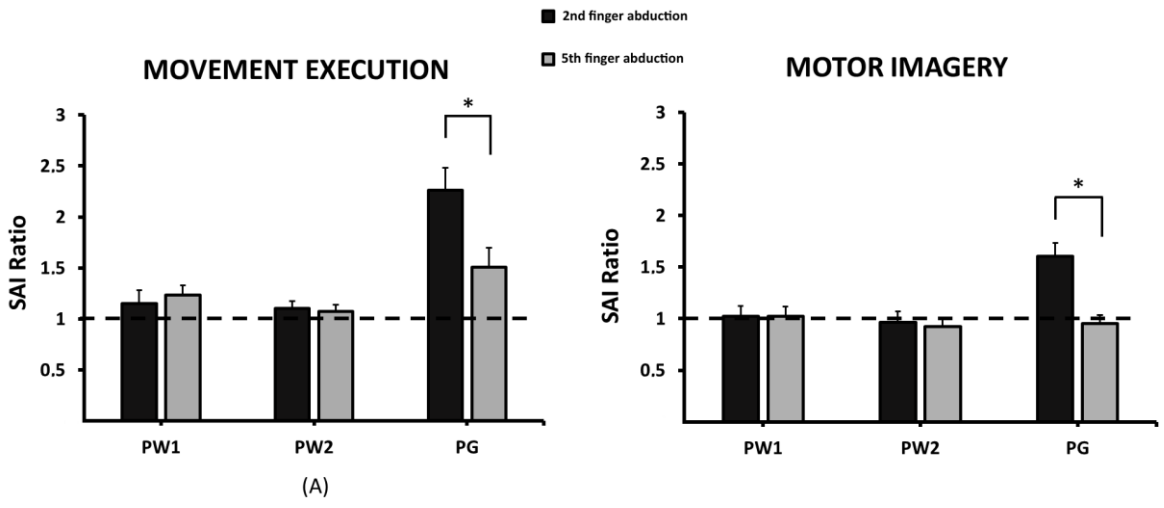


Figure 2. *Short afferent inhibition (SAI) modulation before movement execution and motor imagery.*

Ordinate indicates SAI ratio [SAI ratio = (SAI testing time)/(SAI at rest)]. Values > 1 indicates a reduction of SAI. Vertical bars indicate standard error of the mean (SEM). Asterisks indicate that in both tasks (ME and MI) SAI recorded from first dorsal interosseus muscle (FDI) changed only in the case of homotopic stimulation, i.e. 2nd finger abduction (∗p < 0.05).

Experiment 1: Relationship between sensorimotor modulation during motor imagery and during movement execution

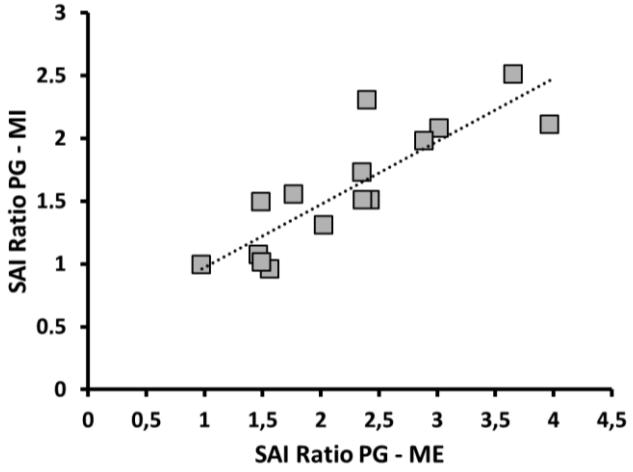
Since sensorimotor modulation (SAI changes) was similar in the ME and MI tasks, we decided to investigate if there was a correlation between SAI modulation (SAI Ratio) during the PG block, when the stimulation was homotopic (2nd finger), during MI and during ME. We found a positive correlation between the two conditions ($r=0.86$, $p<0.001$), displaying that the modulation of SAI inhibitory circuit before movement onset in an imagined task resembles that of the physical execution (Figure 3A). Subjects who had the larger decrease of SAI before ME where the same who had the larger decrease of SAI before MI.

Experiment 1: Relationship between SAI modulation during motor imagery and motor imagery ability

Finally, we wanted to evaluate if SAI modulation (SAI Ratio) during PG block, in the MI condition, correlated with the individual ability to perform motor imagery, that was assessed with KVIQ-10 scale. Particularly, correlation analysis was performed between SAI Ratio during PG block, in the MI condition and the five kinaesthetic items of the KVIQ-10 scale, representing the movement imagined during the experimental paradigm (i.e., finger movements, item #K5), but also movements not involved in the task (i.e., shoulder, #K3; trunk, #K6; hip, #K8 and foot, #K9, movements).

A significant correlation was found only for item #K5, (Rho=0.60, p=0.017) suggesting that the more sensorimotor integration was modulated during MI, the higher was the movement skilfulness to imagine that precise movement (For #K3, #K6, #K8 and #K9, p always > 0.05) (Figure 3B).

A



B

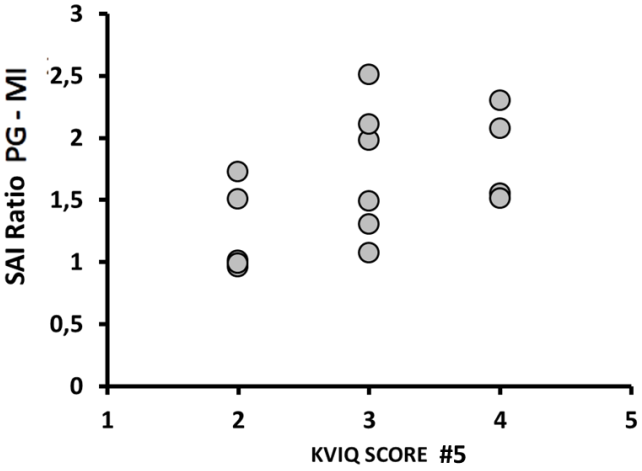


Figure 3. (A) *Correlation analysis between SAI Ratio values during PG block in motor imagery (MI) and movement execution (ME) tasks. There was a significant positive correlation between SAI modulation before the imagined or actual abduction of the 2nd finger (r=0.86, p<0.0001), indicating that the*

higher was the modulation before ME the higher was before MI. ***(B)*** *Correlation analysis between SAI Ratio values during PG block in MI and KVIQ-10 item #K5. There is a significant positive correlation between the modulation before the imagined abduction of the 2nd finger and the ability to kinaesthetically imagine a finger movement (Rho=0.60, p=0.017), indicating that subjects with better imaginative ability had a higher sensorimotor modulation.*

Experiment 2: Effect of PMC anodal tDCS on sensorimotor modulation before MI and ME

For Experiment 2, paired t-test between normalized SAI recorded after REAL and SHAM stimulation did not show any significance (p=0.80), suggesting that a-tDCS did not change *per se* the inhibitory mechanisms of SAI.

Regarding sensorimotor modulation in the planning phase of MI and ME, we found a significant STIMULATION x MOVING FINGER interaction ($F_{1,19}$=6.09; p=0.023) with no significant TASK x MOVING FINGER x STIMULATION interaction. Post hoc analysis showed that when the moving muscle was the 2nd finger (homotopic stimulation), there was a significant difference between REAL and SHAM stimulation (p=0.01), whereas no difference emerged when the moving muscle was the 5th finger (p=0.64). Indeed, when the moving finger was the 2nd finger, sensorimotor modulation at PG testing time increased prior to both the executed and imagined movement after a-tDCS (Figure 4).

Statistical analysis showed also a significant effect of MOVING FINGER ($F_{1,19}$=3.93; p<0.0001) and of TASK ($F_{1,19}$=40.20; p<0.0001), confirming that sensorimotor modulation at PG testing time occurred only when the moving finger was the 2nd finger and was of larger degree prior to ME than to MI.

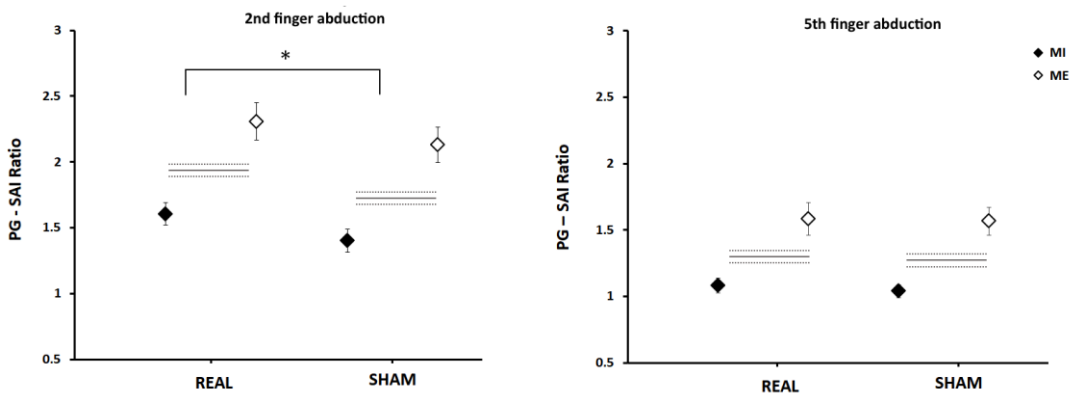


Figure 4. *Sensorimotor modulation modulation recorded post "go cue" (PG) testing time after real (anodal stimulation) and sham transcranial direct current (tDCS) stimulation over PMC during both motor imagery (MI) and movement execution (ME) tasks. Data recorded from first dorsal interosseus muscle during both 2nd finger abduction (homotopic stimulation) and 5th finger abduction (heterotopic stimulation) are displayed. Different stimulations (REAL and SHAM) are indicated on the abscissa. Ordinate indicates SAI ratio [SAI ratio= (SAI testing time)/ (SAI at rest)]. Vertical bars indicate standard error. Horizontal bars represent SAI Ratio mean from MI and ME data whereas dotted horizontal bars indicate standard error mean from MI and ME data. Asterisk indicates that after anodal tDCS, when the moving muscle was the 2nd finger, in both tasks (ME and MI), SAI modulation was higher than after sham tDCS (p=0.01).*

3.2.4 DISCUSSION

The first aim of this study was to investigate sensorimotor modulation before the onset of a mental movement in the task-related muscle and in a surrounding one. Data showed a modulation of somatosensory afferent inhibition just before the expected movement onset, after a "go" cue, similarly to what happened in the planning phase of executed movements, even if to a less extent respect to movement execution. Further, sensorimotor modulation was detectable only in the specific muscle involved in the task and not if the assessed muscle was not involved in the task, for MI as for ME. This first piece of evidence suggests that (i) somatosensory afferent feedback are used during the planning phase of mental movements,

as it happens during executed movements and that (ii) sensorimotor modulation follows the "surround inhibition" mechanism in MI as in ME with selective disinhibition of the cortical representation of the muscle involved in the task.

The second aim of the study was to test whether the magnitude of sensorimotor modulation depended on the level of activity of PMC. To this aim, we artificially increased PMC excitability, through anodal tDCS over PMC, and we tested sensorimotor modulation after the neuromodulation session. Results of the second experiment showed that increasing PMC excitability resulted in an increase of sensorimotor modulation prior to both the executed and imagined movement. This may suggest that the intensity of PMC activity is one of the factors influencing the amount of sensorimotor modulation.

Finally, sensorimotor modulation mechanisms prior to ME and MI likely share overlapping circuits since (i) there is a correlation between individual degree of sensorimotor modulation during ME and MI and (ii) sensorimotor modulation prior to ME and MI is similarly influenced by neuromodulation.

Noteworthy, selective sensorimotor modulation seems to play a role even in motor imagery ability since a correlation was found between the magnitude of sensorimotor modulation and the score at the item of the KVIQ-10 scale that represents the movement imagined during the experimental paradigm (i.e., finger movements).

Sensorimotor modulation during mental movements

Our findings suggest that somatic sensory sensations coming from periphery selectively modulate PMC excitability at the onset of an imagined movement, as it happens during movement execution. Thus, in the process of selection of motor plans, even at the level of

cognitive representation of movement, the cortex makes use of somatic sensory information coming from the periphery. To our knowledge this is the first neurophysiological evidence that such a mechanism exists, and goes along with behavioural results of motor imagery studies[25–27]. Indeed, during mental movements, PMC facilitation occurs more robustly when the hand posture is consistent with the imagined movement than when it is incompatible, suggesting that somatic input from the periphery interact with the motor plan at a cortical level[25,26]. However, to date, there is ambiguity on the role of online afferent feedback. Even if kinaesthetic motor imagery ability is impaired in case of chronic deafferentation, suggesting that somatic inputs are incorporated into the imagery processes[21], during transient deafferentation due to local anaesthesia of the arm, MI processes are slower and less accurate overall, but the influence of biomechanical constraints remained[22].

Here we went further, directly demonstrating that somatic afferent inputs intervene already during the planning phase of mental representation of movement, modulating cortico-spinal excitability. To demonstrate it we used short afferent inhibition, that is mediated at the cortical level through cholinergic-dependent GABA-A receptor activation[28,29] and it is used to probe how sensory input from limbs modulates cortical motor outputs in humans (i.e., sensorimotor integration). The afferent pathway of somatic inputs generating SAI is still debated and either the possibility of a direct arrival of these inputs in PMC from thalamus or an indirect arrival in PMC through thalamic- primary sensory cortex communication have been hypothesized[30,31]. Whatever the case, our neurophysiological study highlights to what extent somatic afferent inputs are exploited during mental representation of movement.

Going deeply, we also demonstrated that somatic afferent inputs exert their modulatory effect in the movement-related muscle, not in the non-involved one, right before an actual or an imagined movement, following a “surround-inhibition” mechanism. Surround

inhibition is a neurophysiological mechanism to focus muscle activity, inhibiting surrounding muscles not involved in the movement. The whole phenomenon of surround inhibition is restricted to the movement initiation phase (just before and during the first phase of EMG onset)[32,33]. There is some evidence that this mechanism is due to active inhibition in PMC by intracortical circuits[32,34] or by and inhibitory network encompassing the prefrontal cortex and subcortical structures[35]. Similarly to what happens during motor execution, reduced corticospinal excitability in those muscles adjacent to the active muscles, but irrelevant to the task, has been showed in a proportion of subjects during mental movements[36]. Further, good imagers demonstrated significantly higher surround inhibition than poor imagers, suggesting that surround inhibition mechanism contributes to accuracy of mental imagery performance[36].

Recent studies highlighted that somatosensory inputs play a role in the regulation of the surround inhibition mechanisms. First, it has been shown that timed sensory feedback from the surround muscle plays a crucial role in adapting surround inhibition for future movements. Indeed, repeated application of a short period of vibration to a surround muscle, that was timed at the onset of the contraction of the muscle involved in the task, increased surround inhibition for a short period after withdrawal of vibration[37]. Second, surround inhibition has been linked to short afferent inhibition, an inhibitory mechanism that is largely dependent from somatosensory afferent information[16,38]. It has been showed that SAI magnitude is reduced in the specific muscle involved in a task not only during tonic and phasic contraction[15,38,39], but also at movement initiation (after a “go” cue)[16].

What is the role of this selective sensorimotor modulation process? Two possible explanations have been taken into consideration. One hypothesis deals with a gating mechanism of the sensory inputs coming from the selected muscle, to reduce noisy afferent

information during planning in order to utilize this information at best during movement for movement feedback correction. However, since we found that this mechanism operates prior to mental movements, that for definition, do not imply movement feedback correction, we think this hypothesis unlikely. The second hypothesis is that sensorimotor modulation contributes in shaping PMC activity in relation to the movement topography, and similarities between ME and MI support this hypothesis. Indeed, a significant correlation was also found between individual degree of sensorimotor modulation before imagined and actual movements onset. Further, our results showed also that sensorimotor modulation processes prior to MI has a functional role. We found a correlation between SAI modulation during imagined movement preparation and the item K#5 of the KVIQ-10 questionnaire. Interestingly the correlation resulted significant selectively for the item pertinent with the movement-involved limb, while for the other parts of the body we did not find any correlation. Thus, MI vividness seems to depend on sensorimotor modulation processes: the more the somatosensory modulation was similar to the actual movement, the more a subject could imagine a kinaesthetic movement of the hand. These results enlarge the idea of the overlapping circuitry that is activated during the planning of an executed and the imagined movement.

Magnitude of sensorimotor modulation depended on the pre-planned level of activity of PMC

We support the hypothesis that somatic afferent input serves to focus PMC activity just before the expected or real movement and that this selective modulation has a functional role on motor imagery ability. However, we also demonstrated that the intensity of PMC activity is one of the factors influencing the amount of sensorimotor modulation. Indeed, by

artificially increasing PMC activity through neuromodulation we were able to increase the magnitude of selective sensorimotor modulation processes. We can speculate that PMC activity may be pre-planned temporally in advance respect to when the somatic afferent input exerts its selective modulatory effect over PMC excitability. This speculation is in accordance with evidence in the literature. It is well-established that PMC activity related to movement preparation occurs, for executed movements, before participants report to be aware of their movement intention[40,41]. Numerous human and animal studies showed that psychophysiological signals of intention to move, such as the contingent negative variation (CNV) and changes in the μ-band and β-band EEG power, occur in a pre-cueing task like the one adopted in this study, already during the fore period between the warning and the go signals[42,43]. Related to our experimental paradigm, this time interval refers to post-warning assessments, before the occurrence of sensorimotor modulation prior to executed and imagined movement (occurring only at the post-go testing time).

Interestingly, this observation has been replicated for mental movements, with a difference of signal amplitude between the preparatory phase of ME and the one of MI on the CNV, the activation being more important during ME[44,45]. Thus, a functional difference mainly consisting of quantitative modulations of the activity of PMC is likely to be already present in the process involved when a subject prepares to execute or prepares to imagine a motor act[45]. In support to this, it has been shown that a startling acoustic stimulus, if delivered from 1500 to 200 ms prior the movement onset, evokes a premature release of the planned movement for an executed movement, but not for an imagined one[46].

Our findings, related to the possibility to increase sensorimotor modulation prior to an imagined task, by increasing PMC activity, could explain why, by increasing PMC activity prior MI training, it is possible to increase the efficiency of MI training. Indeed, active

excitatory tDCS significantly enhanced the motor-imagery-induced improvement in motor function as compared with sham tDCS[47]. There was a specific effect for the site of stimulation such that effects were observed after PMC stimulation in different studies[47–49]. Here we observed that the degree of sensorimotor modulation correlates with accuracy of mental movements and we could thus hypothesize that excitatory neuromodulation over PMC might be able to induce a better motor imagery performance through fostering sensorimotor modulation processes. However, this is only a hypothesis that deserves to be addressed in future studies. Further, it is worthy to underline that other factors beyond PMC activity could theoretically influence the degree of sensorimotor modulation process. We specifically refer to the amount or specificity of sensory inputs[16] or to the complexity of the pre-planned task[50]. These other factors should also be addressed in future studies.

Conclusions

Here we showed for the first time that sensorimotor modulation operates during the cognitive representation of movement in a very specific way as it operates during movement execution. Further, this modulatory process is restricted to the movement initiation phase, and is at least partially dependant on the amount of pre-planned PMC activity.

This piece of information can give new insights when adopting cognitive strategies to foster motor performance in the field of rehabilitation or in sport.

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3.3 LONG-TERM RETENTION OF MOTOR SKILL AFTER MOTOR IMAGERY TRAINING

3.3.1 INTRODUCTION

In our daily life to learn a movement is a complex but unconscious process that makes an action, like cycling, skiing or playing an instrument easier and easier to perform as time passes. Complex motor tasks are learned through repetition and training, which results in lasting improvement in the temporal and spatial accuracy of movements[1,2].

In a laboratory setting, it is possible to study motor learning through a common experimental paradigm, that measures the incremental acquisition of movements into a well-executed behaviour, namely motor sequence learning.

According to Doyon and Benali[3], the cognitive processes and the neural substrates that mediate our capacity to learn follow five distinct phases: a “fast (early) learning stage”, that occurs within a single training session; a “slow (later) stage” where further improvement takes place after several sessions of practice and a “consolidation stage”, in which after some hours from the last training a performance consolidation or further improvements can be recorded. When consolidation has occurred, cognitive resources are less needed in the “automatic stage”. The final stage of motor learning is the “retention stage”, when the movement is effortless and can be easily executed after a long period.

The learning of a motor skill is commonly attained via physical repetition of the skill[4]. However, research has shown that cognitive training, such as motor imagery (MI) and action observation training, can also be applied to effectively facilitate skill learning, either alone, or combined with physical practice[5].

MI is a dynamic state during which motor actions are mentally simulated, without actual movement[6,7]. Motor imagery training has been used as an effective resource to facilitate motor learning[8–10]. Indeed, meta-analyses on this topic have reported that motor imagery training has a positive effect on motor performance, even though the degree of its effectiveness varies with some factors, such as type of task, experience level of participants or duration of practice[11]. Furthermore, motor imagery training has been shown to be more effective compared to no practice in inducing motor performance improvements, but less effective than physical practice itself[12]. This behavioural observation was accompanied by the neurophysiological finding that even if both one training session based on MI or physical practice led to the development of neuroplasticity, effects after physical practice were stronger than after motor imagery practice[10]. One possible explanation deals with different sensorimotor mechanisms operating during the two training methods. Indeed, one of the main differences between physical and motor imagery practice is the lack of somatosensory afferent inputs in the imagined movements. According to this hypothesis, we recently showed that provision of somatosensory inputs during a single session of motor imagery training enhances learning-induced plasticity in human motor cortex[12].

Nonetheless, the limit of the studies present in the literature is that they deal only with the first stages of motor learning process and no data are available related to long-term retention of motor skill after motor imagery training.

The aim of this study was to observe if and how a training based on the cognitive representation of movement and characterized by with lack of sensory feedback from body motion and the environment, i.e. MI, differently affected the phases of motor learning, in respect to movement execution (ME). We hypothesize that some differences would emerge

between MI and ME training in consolidation and retention of motor skill, likely dependant on the different sensorimotor mechanisms operating during the different trainings.

3.3.2 MATERIALS AND METHODS

Subjects

Ten right-handed subjects were recruited for the experiment (mean age 24.33 ±5.6, 4 males). They were divided into two groups, of 5 subjects each, performing MI or ME training. All subjects were in good health, without any nervous, muscular, orthopaedic or cognitive disorders. Right arm dominance was determined by means of the Edinburgh Handedness inventory[13]. In order to assess subject's imagination vividness, we administered the "Kinaesthetic and Visual Imagery Questionnaire" (KVIQ-10), a 10 item questionnaire that evaluates visual and kinaesthetic imagery scale in five different movements. Rating consists of a 5-point ordinal scale, where the higher is the score the higher the intensity of the sensations associated with the imagined movement or the clarity of the visual image. The experimental protocol was approved by the ethics committee of the University of Genoa and was carried out in agreement with legal requirements and international norms (Declaration of Helsinki, 1964).

Experimental design

Participants trained and their performance was assessed over a period of 15 days. Figure 1 outlines the number of assessment and training sessions performed each day. Each training

session lasted about 10-15 minutes, however the time reduced with learning. In the first two days, subjects trained once per day (Tr1 and Tr2). Motor performance was evaluated before training (Baseline), and 5 minutes immediately after the end of each training session (Tr1+5', Tr2+5'). Short-term retention was assessed after 2 hours (Tr2+2h). We defined this block as "the first learning phase". On Day 3 participants did not train, but motor performance was assessed (Tr2+1d) to obtain a second retention measure of the initial learning. On Day 4 and on day 5 training and assessment sessions were repeated in the same fashion as on Day 1 and 2. We defined this block as "the second learning phase". Finally, to evaluate the "retention" of motor skill learning participants performed further assessment tests on Day 8 and on Day 15.

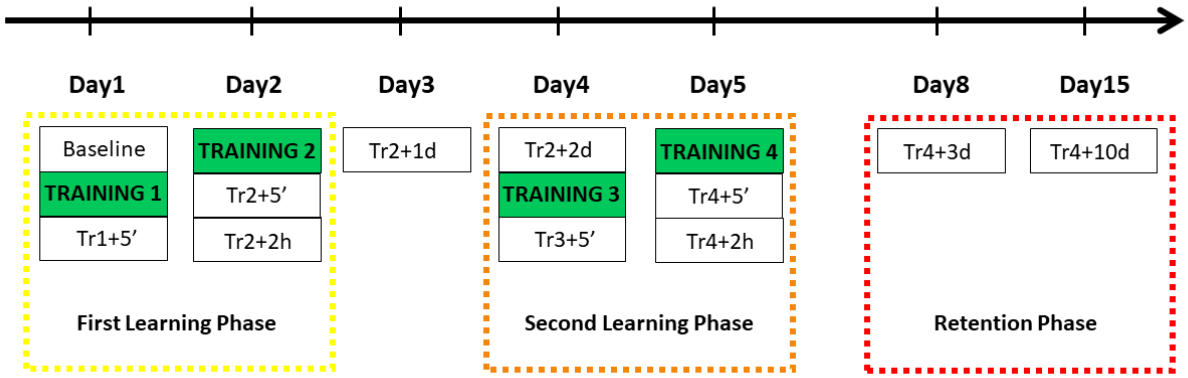

***Figure 1**. Experimental protocol. Subjects trained in four separate sessions, both in the ME and MI groups. Each training consisted in 10 blocks of 4 repetitions of an 8-finger touches sequence (320 finger movements). Motor assessments (white blocks) were performed before, immediately after training (5 minutes and 2 hours) and during retention. Assessment consisted in one single block of 4 repetition sequences (32 finger movements).*

Behavioral assessment and training

Subjects were seated on a chair wearing sensor-engineered gloves (Glove Analyzer System (GAS), ETT S.p.a., Italy) on both hands. We choose an eye-close paradigm to avoid confounding effects due to different kind of training (ME, MI). The basic unit of the behavioural task, used both for the assessment and the training sessions, consisted of an eight finger movements sequence (for ME 4, 1, 3, 2, 4, 2, 3, 1; for MI 3, 1, 4, 2, 3, 2, 4, 1).

Training. All the participants had a short familiarization session during which they had to perform few trials of the task at a natural velocity. After 3–5 finger sequences, all participants reported being comfortable with the task. Then, ME training consisted in performing as fast as possible 10 blocks of 4 sequences with 10 s rest between the blocks (total number of finger movements: 320 (8 fingers × 4 sequences × 10 blocks). The MI practice consisted in imagining the same task, with a different fingers sequence, but of similar complexity. Precisely, participants were asked to imagine themselves performing the movement ("kinesthetic imagery" or first-person perspective), as they would actually do (Gentili et al., 2010). They were asked to feel the motion of their fingers and the contact between the distal phalanx of the thumb and those of the other fingers.

Assessment. To assess motor performance, participants were asked to execute 1 block of 4 sequences, as fast as possible, with the right hand, on different testing times before or after the practice sessions (see Experimental design).

Statistical analysis

Because of the limited sample size, as a preliminary data analysis, we decided to perform the statistical analysis separately for the two groups, ME and MI.

Data collected with the sensor-engineered glove were processed with a customized software. Finger opposition movements were described by movement rate, i.e. the number of contacts per second (Hz), calculated from the finger touches of the corrected sequences.

We checked that all variables were normally distributed (Shapiro-Wilk W test) and that sphericity was respected (Mauchly tests). For each motor learning phase, 1st learning, 2nd learning and retention, we performed a repeated measures Analysis of Variance (RM-ANOVA). This analysis was preliminary applied to raw motor performance data (Movement RATE).

For the 1st learning phase behavioural data were entered in a RM- ANOVA with TIME (Baseline, Tr1+5', Tr2+5', Tr2+2h, Tr2+1d) as a within- subject factor. For the 2nd, slow, learning phase behavioural data were entered in a RM- ANOVA with TIME (Baseline, Tr2+2d, Tr3+5', Tr4+5', Tr4+2h) as a within- subject factor. Lastly, we evaluate retention phase with the same ANOVA with TIME (Baseline, Tr4+5', Tr4+3d, Tr4+10d) as a within-subject factor. Statistical analysis was run separately for data collected from MI or ME groups. Statistical analysis was performed with SPSS 22.0. P-values of 0.05 were considered as threshold for statistical significance.

3.3.3 PRELIMINARY RESULTS

First learning phase. The number of finger movements / min increased from Baseline to the end of this phase in both ME and MI groups. Accordingly, RM- ANOVA revealed a significant effect of TIME for ME ($F_{1,4}$= 4.93, $p<0.05$) and for MI ($F_{1,4}$=7.35, $p<0.05$). Post hoc analysis highlighted that while the movement rate recorded 1 day after the second training session

(Tr2+1d) was significantly higher than movement rate recorded 2 hours after the second training session (Tr2+2h) (p=0.03) in the ME group, no differences emerged in the MI group between movement rate recorded at Tr2+2h and that recorded Tr2+1d (p=0.96).

Second learning phase. We found similarities between the two groups also in the second learning phase. Indeed, RM- ANOVA revealed, a significant effect of TIME for ME ($F_{1,4}$=12.22, p<0.05) and for MI ($F_{1,4}$=22.32, p<0.05). However, post hoc analysis highlighted that in the ME group, apart a significant difference with baseline (baseline *vs* Tr2+2d, Tr3+5', Tr4+5', Tr4+2h, p always <0.01), no differences emerged between the different testing times (Tr2+2d, Tr3+5', Tr4+5', Tr4+2h). In the MI group, instead, apart a significant difference with baseline (baseline *vs* Tr2+2d, Tr3+5', Tr4+5', Tr4+2h, p always <0.05), movement rate recorded 5 minutes after the fourth training session (Tr4+5') was significantly higher than both that recorded at the first assessment of the second learning phase (Tr2+2d) (p=0.015) and that recorded at the last assessment of the second learning phase (Tr4+2h) (p=0.04).

Retention. The last analysis showed once more a shared effect of TIME in both training groups [for ME ($F_{1,3}$=30,15, p<0.05) and for MI ($F1_{,3}$=45.39, p<0.05)]. Post hoc analysis showed that, only in the MI group, movement rate recorded 3 days and 10 days after the last training session (Tr4+3d and Tr4+10d) was lower than movement rate recorded immediately after the last training session (Tr4+5') (p=0.07 and p=0.021 respectively).

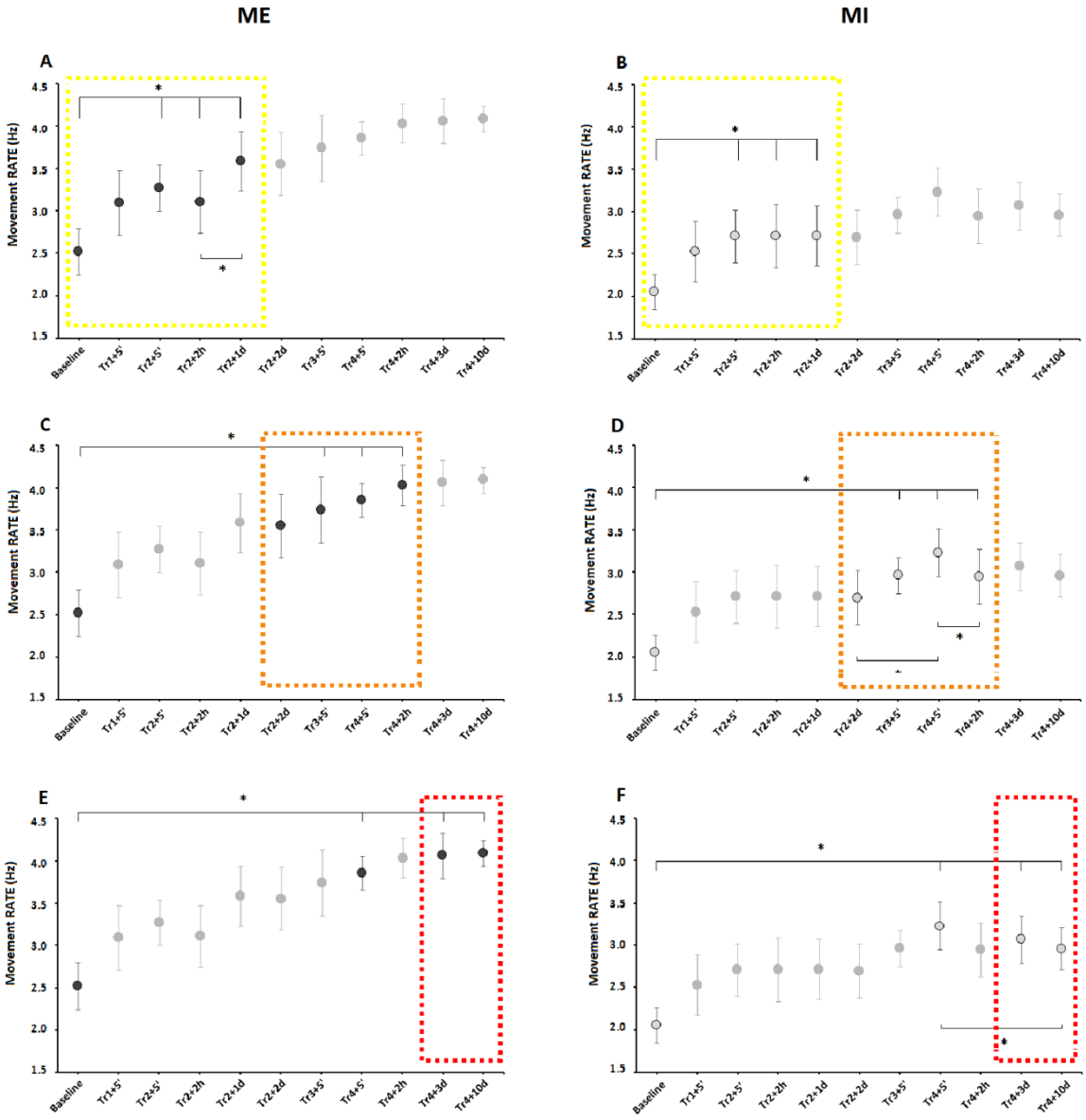

Figure 2. *Panels on the left side (A, C, E) represent finger movement RATE changes during the process of sequence learning collected from the ME group, while panels on the right side (B, D, F) represent data from MI group. In each chart, assessment scores used for statistical analysis are highlighted. Coloured dotted boxes define the different learning phases. A and B represent the first phase, C and D the second, E and F represent retention phase. Vertical bars indicate standard error of the mean (SEM). Asterisks indicate the level of significance (*p < 0.05).*

3.3.4 DISCUSSION

Preliminary results of this study describe an improvement in terms of movement execution velocity, in both groups, during the days. This suggests that both MI and ME training induced motor learning, similarly with what reported in previous studies[10,12,14]. The novelty of this study is the repetition of the training and assessment sessions through 15 days that allows us to test for long-term retention of motor skill after motor execution and motor imagery training. Following our a-priori hypothesis, some differences emerged in the consolidation and retention of motor skill after MI or ME training.

The first learning phase results are somehow expected, as they confirmed the results of a previous study by our group[12]. The maintenance of the acquired skill, tested as movement rate on the day subsequent the training session was present in the ME group, where we recorded an additional increase in movement rate, but not in MI group, where, after the improvement recorded 2 hours after the training session, no further increase of movement rate was observed 1 day after the training session.

The second learning phase gave us some novel information. The ME group presented a linear increase of movement rate through the third and the fourth training sessions at all the testing times, included those temporally far from the training session, as the Tr4+2h. In the MI group, instead, we found an increase of movement rate immediately after the fourth training session (Tr4+5'), that was lost two hours later.

Finally, the last analysis, on the retention phase, marked again the inability to fix on the long term the skill acquired through mental repetition, as the improvement achieved after the last training was slowing reducing one and two weeks after. Differently, in the ME group, performance improvements were retained.

As we know, ME and MI lays on different sensorimotor mechanism during training. MI, without any bottom-up feedback, can only rely on internal forward models (that mimic the causal flow of the physical process by predicting the future sensorimotor state)[15] to provide state estimation needed during the training, being presumably less accurate and less precise than during physical training. On the other hand, ME, involving sensory and motor processes can provide accurate and precise state estimation, in order to improve velocity and quality of the movement.

MI training is able to induce motor learning, each time after a training session, but this movement improvement, likely because of the lack of the sensory feedback, can't be fixed over time.

In conclusion these preliminary results emphasize the role of the somatosensory inputs on motor learning, particularly in the retention phase.

As a preliminary study we need to implement the sample size and to focus our attention on the different kinematic properties of finger movements, as the time dedicated to movement and the time dedicated to the touch between fingers might change differently. Further, to better understand the neurophysiological mechanisms at the basis of these findings, it would be of great interest to follow cortical excitability changes over the different phases considered by this experimental design. Finally, to confirm the role of the sensory feedback on motor skill retention, it would be useful to test the effect of training based on the combination of sensory feedback and motor imagery.

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CHAPTER 4. MOVEMENT ILLUSION IN REHABILITATION FIELD

4.1 MIRROR VISUAL FEEDBACK TO IMPROVE BRADYKINESIA IN PARKINSON'S DISEASE

4.1.1 INTRODUCTION

Bradykinesia is one of the cardinal symptoms of Parkinson's disease (PD) and is defined as reduced speed when initiating and executing a single movement with progressive reduction of its amplitude, up to complete cessation during repetitive simple movements[1,2]. PD motor manifestations, including bradykinesia, can begin unilaterally, typically in one limb segment, when dopamine concentrations fall below 60–70% in the contralateral striatum[3]. Throughout the course of the disease the asymmetry of major features persists in most cases; asymmetric onset has even been proposed as a criteria for PD diagnosis. Generally, bradykinesia is experienced in repetitive/rhythmic voluntary movements such as finger tapping or steady gait, thus inducing motor difficulties in PD that affect almost all daily life activities[4–6]. Furthermore, PD patients may exhibit greater impairment in the speed rather than in the amplitude of movement (or vice versa), and these different phenotypes may differently respond to pharmacological treatment[7].

Beside conventional rehabilitative approaches, action observation (AO) has recently been suggested as a novel technique to improve bradykinesia in PD[8], with positive effects on the spontaneous rate of finger movements even after a single session of AO training[8].

Mirror visual feedback (MVF) therapy is an innovative rehabilitative approach, which attracted a growing interest in the last few years (for a review see[9]). MVF therapy aims at supplying visual feedback from the affected arm in a very peculiar way, as the subject is performing the action with the opposite non-affected limb (thus receiving motor training and proprioception from that limb), but receives visual feedback from the affected limb[10]. MVF was originally used to alleviate phantom-limb pain after upper limb amputation[11]. Since then, the technique has been successfully applied to improve upper limb function in other neurological diseases[12,13], and in the elderly[14]. Data from the literature suggest that MVF combined with motor skill training may improve performance of the trained and untrained hand, most likely inducing plasticity modifications in both the primary motor cortices[15,16].

The above-mentioned evidence paved the way for the use of the MVF therapy as a therapeutic option for treating bradykinesia in PD.

Indeed, one interesting feature of the MVF is that only one hand needs to be actively trained to provide performance improvement of both the trained and untrained hand[15,17]. In PD patients with bradykinesia, the untrained hand should be on the most affected side, in which the ability to undertake sequential or simultaneous movements is severely reduced[18]. In this way training the less affected hand can improve the function of the other, more affected, hand. Further, it is worth noting that the relationship between fatigue and bradykinesia in PD is still under discussion. Fatigue is a common symptom in PD, with a reported prevalence of approximately 33%[19]. Fatigue could interfere with the outcome of a rehabilitative program[20], and therefore, assuming that a relationship between bradykinesia and fatigue exists, training the less affected side may improve the training outcome. Finally, from a neurophysiological point of view, a large body of evidence suggested that hypoactivation of PMC could be the functional correlate of PD bradykinesia[21–23] .

Hence, the present study was designed (i) to investigate whether MVF therapy can influence specific aspects of bradykinesia, such as speed, (ii) to explore whether MVF was able to induce excitability changes in PMCs in PD as already reported in healthy controls (iii) to elucidate whether the severity of fatigue might influence changes in motor performance induced by MVF in the trained and untrained hands.

To this end, participants underwent a 'mirror training', performing a sequential finger motor task with the less affected side for PD patients (dominant/right hand for healthy subjects (HS)) outside a mirror box. At the same time, all participants were required to carefully observe hand's movements in the mirror, in order to create the illusion of moving the more affected hand in PD patients and the left (non-dominant) hand in healthy controls, thus creating visual feedback training[10]. Motor performance and cortical excitability of PMCs were tested before and immediately after MVF training. Further, in patients with PD the extent of perceived fatigue was evaluated by means of the Parkinson's Fatigue Scale.

Our hypotheses were the following: (i) MVF would induce behavioural improvements and cortical excitability changes in the untrained side in both healthy subjects and PD patients and (ii) the extent of fatigue perceived by PD patients may have a greater influence on the behavioural improvement in the trained hand compared to the untrained hand.

4.1.2 MATERIALS AND METHODS

Study design and participants

Thirty-three participants (21 patients with Parkinson's disease (PD) and 12 healthy subjects (HS)) were recruited for this study. Twelve PD and twelve HS were involved in the main experiment (MVF training), while nine additional patients with PD were recruited for taking

part in a control experiment (training without MVF). Informed consent was obtained from all participants according to our institution's policy and to the Declaration of Helsinki. The study was approved by the local ethics committee of the University of Genoa. All patients with PD (12 females and 9 males; age, 58 - 80 years; mean age 72.10 ± 4.89), diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank criteria, were recruited from the outpatient Movement Disorders Clinic of the University of Genoa. All patients were in Hoehn and Yahr stages 1 to 3 and on a stable medication regimen. Disease severity was determined by means of the Unified Parkinson Disease Rating Scale (MDS-UPDRS; Part III Motor). The following exclusion criteria were applied: (1) past history of neurological conditions other than PD, (2) deep brain stimulation, (3) Mini-Mental State Examination score <24, (4) visual field defects, that could prevent subjects from seeing their hand reflection, and (5) severe orthopaedic problems of the upper limb. To assess bradykinesia severity we used 'The Modified Bradykinesia Rating Scale'[24]. All patients suffered from more severe symptoms on one side of their body at the time of symptom onset, and at the time of enrolment in this study. This side is referred to as the more affected side. In each single patient the designation of "more affected side" was determined from the clinical history and confirmed by clinical evaluation.

The Parkinson's Fatigue Scale (PFS-16), a full Likert 16-items scale, was used to evaluate the extent of perceived fatigue[25] . Rating is based on feelings and experiences over the prior 2 weeks and scoring options for each item range from 1 ("strongly disagree") to 5 ("strongly agree"). A total PFS score is calculated as the average item score across all 16 items and ranging from 1 (minimum) to 5 (maximum).

A total of twelve age and gender matched healthy subjects (HS) (6 females and 6 males; age, 64-76 years; mean age 71.5 ± 3.89) with normal neurological examination and no history of

neurological disorders were recruited from the hospital staff or relatives of the patients. None of the HS had orthopaedic hand impairment or visual field defects.

All participants enrolled in this study were right-handed based on Edinburgh Handedness Inventory[26] and had no contraindication to TMS. A written informed consent was obtained from all participants. Detailed information of demographic and clinical features of all patients is shown in Table 1.

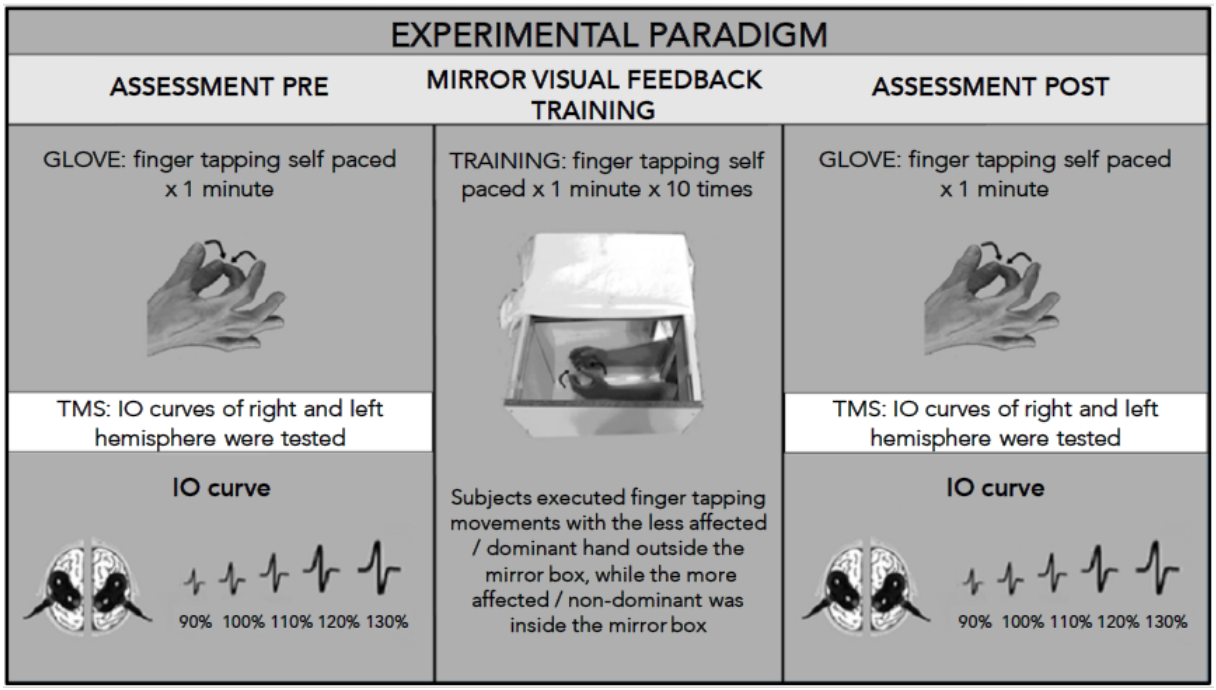

Figure 1. *Experimental paradigm.*

Table 1. Demographics and clinical characteristics of PD patients

Group	Patient	Age (years)	Gender	H&Y	UPDRS III (score)	MBRS (untrainedhand)	MBRS (trainedhand)	PFS-16
M	1	58	F	1.5	13	3	0	2.63
M	2	80	M	3	34	8	6	3.63
M	3	68	F	3	31	5	2	2.69
M	4	73	M	2	34	2	1	2.31
M	5	69	M	3	51	8	6	2.13
M	6	75	F	3	20	3	1	2.81
M	7	71	F	2.5	23	4	2	2.06
M	8	72	F	2	12	6	4	2.63
M	9	74	M	2.5	21	4	2	1.75
M	10	75	M	3	27	4	3	2.06
M	11	66	F	2	16	4	3	2.50

M	12	74	F	2.5	35	7	5	3.44
	Mean	**71.25**		**2.5**	**26.42**	**4.83**	**2.92**	**2.55**
C	1	68	M	2	23	3	2	2.25
C	2	72	F	2	15	4	2	1.25
C	3	80	F	2.5	37	8	2	1.75
C	4	70	M	2	29	7	5	1.81
C	5	72	F	2	12	6	4	2.63
C	6	74	F	2.5	35	7	5	3.44
C	7	78	F	2	28	6	3	2.06
C	8	71	M	2	23	7	5	1.63
C	9	74	M	2.5	27	5	3	2.19
	Mean	**73.22**		**2.16**	**25.44**	**5.88**	**3.44**	**2.11**

PD, Parkinson's Disease; UPDRS III, Unified Parkinson's disease rating scale; H&Y, Hoehn and Yahr stage; MBRS, Modified Bradykinesia Rating Scale; PFS-16, Parkinson Fatigue Scale.

Experimental paradigm

Motor performance and cortical excitability were evaluated before and immediately after MVF training in the main experiment and training without MVF in the control experiment. The experimental paradigm is shown in Figure 1.

Main experiment - MVF training

Motor training was performed with the use of a mirror box. A plastic collapsible triangular box with a mirror (38 cm long, and 22 cm high) attached on one side was placed on the table so that the mirror would reflect one of the subject's hands while the box hid the other from subject's view. The box had open ends to allow subjects to insert their hands[27]. Subjects were asked to hide their more affected (for PD) / non-dominant (for HS) hand behind the mirror. The motor task consisted of ten sessions (one minute each) of finger opposition movements with the less affected / dominant hand with MVF superimposed to the other hand. To avoid fatigue, every session was alternate with one-minute rest interval.

Control experiment - Training without MVF

Nine additional PD patients (Control PD group) were enrolled in this control experiment. This experiment was planned to quantify the extent to which performance improvements and excitability changes in the untrained side may have occurred as a result of training of the contralateral hand by means of a mechanism of intermanual transfer. Participants were asked to place both arms inside the mirror box and to perform the same motor training using the less affected hand as in the main experimental condition. The mirror was covered with a black plastic board and participants were required to carefully watch the trained hand during training session.

Motor assessment

The motor task consisted in the execution of repetitive finger opposition movements (opposition of the thumb to index, middle, ring and little finger), for 1 minute at their spontaneous velocity with both hands, one at a time, in a random order. Motor performance was recorded by means of a sensor-engineered glove on both hands (Glove analyzer System [GAS], eTTSpA., Italy) and data were acquired at 1 kHz (National Instrument board 800008B-01).

The main outcome measure was the number of self-paced finger movements that participants were able to execute in 1 minute, whereas kinematic parameters (i.e. inter-tapping interval, touch duration, and percentage of correct sequences) were secondary outcome measures.

Data from glove were processed with a customized software (GAS, ETT, S.p.A., Italy) and the following parameters were computed: (i) the Inter-Tapping Interval (ITI), defined as the time interval between the end of a thumb-to-finger contact and the beginning of the subsequent

contact in the finger motor sequence; (ii) the Touch Duration (TD), the contact time between the thumb and another finger; and (iii) the Movement Rate calculated as [1/ (ITI + TD)]*1000 and expressed in Hertz. The number of self-paced finger movements was calculated by multiplying the Movement Rate (that express the number of finger touches in one second) for 60 seconds, that is the duration of the entire task. Moreover, we quantified the learning effect by measuring the increase in the number of self-paced finger movements in the assessment after MVF training with respect to before MVF training (Δ score of the number of fingers movements: number of finger movements/min after MVF training - number of finger movements/min before MVF training).

Finally, spatial accuracy (i.e., the ability to correctly execute the finger sequence) was investigated by calculating the percentage of correct sequences (% CORR_SEQ). The uncorrected sequences were discarded from further analysis.

In the Control Experiment (Training without MVF), gain in motor performance of the untrained hand was quantified in the Control PD group by measuring the increase in the number of self-paced finger movements in the assessment after training with respect to before training (Δ score of the number of fingers movements: number of finger movements/min after training - number of finger movements/min before training).

Cortical excitability

Electromyographic (EMG) activity was recorded from the right and left first dorsal interosseous (FDI) muscles, with silver disc surface electrodes. The ground electrode was placed at the wrist. EMG signals were amplified and filtered (20Hz to 1kHz) with a D360 amplifier (Digitimer). The signals were sampled at 5000 Hz, digitized with a laboratory interface (power1401; Cambridge Electronics Design), and stored on a personal computer

for display and later offline data analysis. Each recording epoch lasted 400 ms; 100 ms preceded the TMS stimulus. Trials with background EMG activity were excluded from analysis.

TMS was performed with a single Magstim 200 magnetic stimulator (Magstim Co., Whitland, Dyfed, UK). We determined the optimal position for activation of the left and right FDI muscles by moving the coil in 0.5 cm steps around the presumed motor hand area (referred to as 'motor hot spot'). The figure-of-eight coil (wing diameters, 70 mm) was placed tangentially to the scalp with the handle pointing backward and laterally at 45° to the sagittal plane inducing a posterior anterior current in the brain. The 'motor hot spot' was marked with a red wax pen by drawing a semilunar line following the anterior bifurcation of the coil and a straight line indicating the orientation of the coil handle.

At the beginning of the experiment, the stimulus intensity needed to evoke MEPs of approximately 0.8−1 mV peak-to-peak amplitude was defined (S1mV). Cortical excitability of both the left and the right PMCs was tested by means of TMS Input-Output (IO) recruitment curve. During the IO curve the intensities of single TMS stimuli were expressed as a percentage of S1mV intensity. Twelve MEPs were recorded with 90%, 100% (S1mV), 110%, 120% and 130% stimulus intensities. For each participant, the peak-to-peak MEP amplitude on single trials was used to calculate the mean MEP amplitude at each stimulus intensity. Intensities were random presented, in order to minimize hysteresis effects[28].

Statistical analysis

We checked that variables were normally distributed (Shapiro-Wilk W test) and that sphericity was respected (Mauchly tests). To evaluate motor performance improvement, the mean values of the number of finger movements/min, ITI, TD, and number of correct

sequences were submitted to a repeated-measures ANOVA (RM-ANOVA) with Time (before and after MVF training) and Hand (trained and untrained) as within subjects factors and Group (PD and HS) as between subjects factor. Increase in the number of finger movements/min gained after MVF training in PD and HS (Δ score of the number of fingers movements) was compared by means of a RM ANOVA with Hand (trained and untrained) as within subjects factor and Group (PD and HS) as between subjects factor. Furthermore, to investigate a possible relationship between training-induced behavioral improvement and the severity of the symptom fatigue (PFS-16 score), the correlation between the Δ score of the number of fingers movements and the PFS-16 score was analysed with Spearman's correlation coefficient. This analysis was performed for the trained and untrained hands separately.

To evaluate the effect of MVF training on IO curves, data were subjected to a RM ANOVA with Time (before and after MVF training), Hemisphere (Trained and Untrained) and TMS Intensity (90%, 100%, 110%, 120%, 130%) as within subjects factors and Group (PD and HS) as a between- subjects factor.

To test whether the training effect on motor performance and corticospinal excitability of the untrained side could be attributed to MVF, data from Mirror and Control PD groups were compared. The Δ score of the number of fingers movements of the untrained hand in the Mirror PD group was compared with that obtained in the Control PD group by means of the unpaired Student's t test. IO curves data obtained from the untrained hemisphere were compared by means of RM-ANOVA with Time (before and after training) and TMS Intensity (90%, 100%, 110%, 120%, 130%) as within subjects factors and Group (Mirror PD and Control PD) as a between subjects factor.

P-values of 0.05 were considered as threshold for statistical significance. *Post hoc* analysis of significant interactions was performed by means of t-tests applying the Bonferroni correction for multiple comparisons when necessary. Statistical analysis was performed with SPSS 22.0.

4.1.3 RESULTS

Motor performance

The number of finger movements/min increased in both the trained and untrained hand after MVF training in PD patients as well as in HS (Figure 2). Accordingly, RM ANOVA revealed a significant effect for Time ($F_{(1,22)}$=14.36 p=0.01). No significant interaction was found for Time*Group, Time*Hand, or Time*Hand*Group (p always >0.05). As expected, a significant effect of Group ($F_{(1,22)}$=3.78, p=0.045) was found, showing that PD patients executed a lower number of finger movements respect to HS.

Due to the fact that no significant changes were found in Touch Duration (Figure 2) (RM-ANOVA: p always < 0.05), the increased number of finger movements/min could be ascribed to the reduction of the movement time (ITI) (Figure 2). After MVF training, the mean value of ITI was significantly reduced (Time, $F_{(1,22)}$=11.98, p=0.002) with no differences between groups (PD and HS) and between the trained and untrained hands (post hoc analysis Time*Group, Time*Hand, or Time*Hand *Group, p always >0.05). Statistical analysis also showed that ITI was longer in PD patients respect to HS (Group, $F_{(1,22)}$=5.89, p=0.024). However, this result was mainly due to a longer value of ITI observed in the untrained (more affected) hand of PD patients respect to HS. Indeed, RM-ANOVA showed a significant

Hand*Group interaction ($F_{(1,22)}$=4.96, p=0.036) and post hoc comparison revealed that ITI was significantly longer in the untrained hand in PD patients than in HS (p=0.018).

At the end of the experimental protocol, the number of correct sequences increased only in the trained hand (Figure 2). Post hoc comparison on Time*Hand interaction ($F_{(1,22)}$=7.22, p=0.01) showed a significant increase of the number of correct sequences in the trained with respect to the untrained hand (p= 0.019) after MVF training. Overall, the number of correct sequences was lower in PD than in HS participants (RM-ANOVA: Group, $F_{(1,22)}$ =5.81, p=0.025) with a significant Hand*Group interaction ($F_{(1,22)}$=4.56, p=0.04). Post hoc analysis showed that the number of correct sequences was lower in PD with respect to HS in the untrained (more affected) hand (p=0.006), but not in the trained one (PD *vs* HS, p=0. 29).

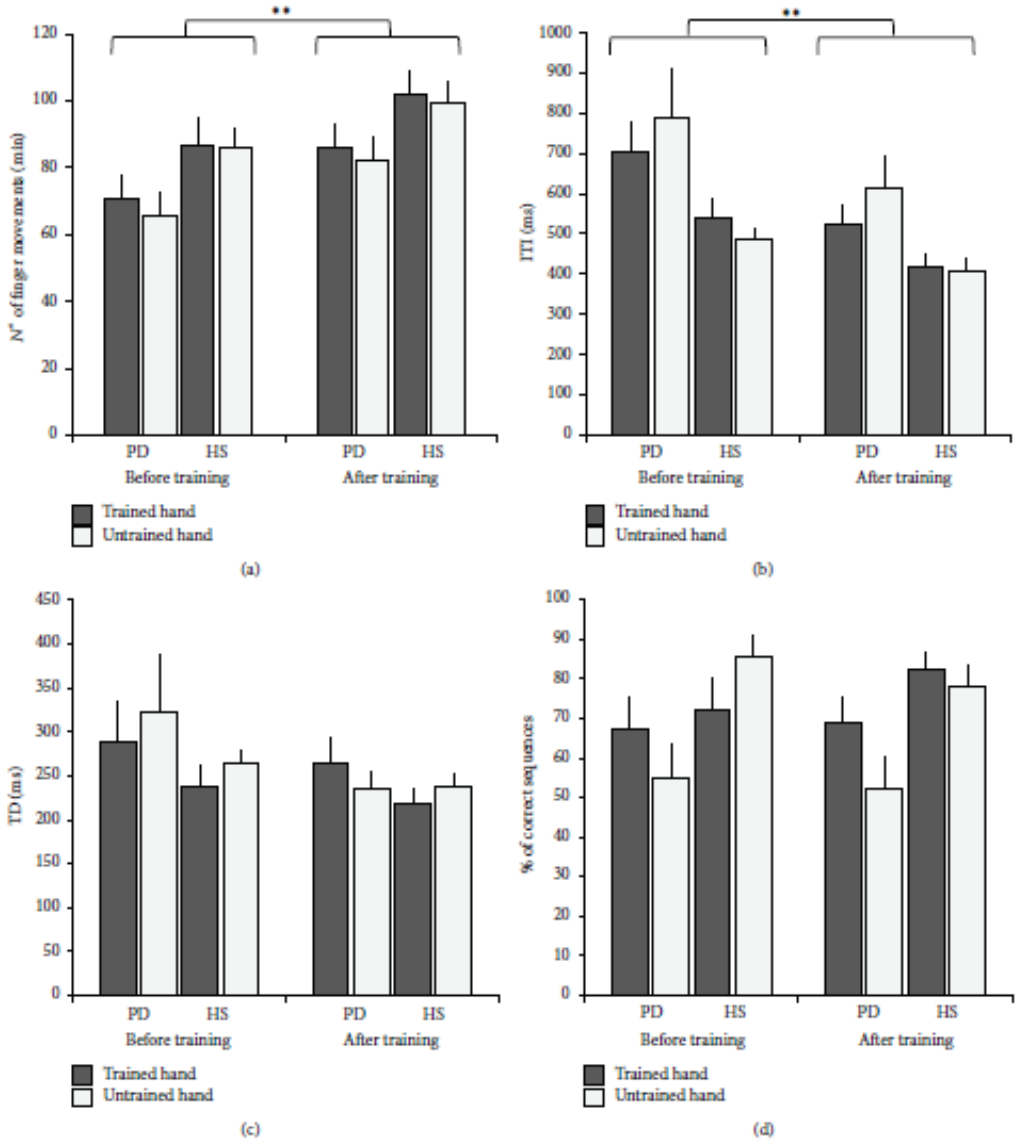

Figure 2.*Mirror visual feedback (MVF) training effect on behavioural data. Groups [Parkinson's disease (PD) patients, Healthy Subjects (HS)] and hand (trained, untrained) are indicated in the abscissa. Data recorded at baseline (before training) and after MVF training session are reported. Ordinate indicates the mean values of* ***(A)*** *number of finger movements performed in one minute during the assessments;* ***(B)*** *intertapping intervalexpressed in milliseconds;* ***(C)*** *touch duration expressed in milliseconds; and* ***(D)*** *% of correct sequences. Vertical bars indicate standard error of the mean (SEM). Asterisks indicate that in both groups the number of finger movements performed in one minute significantly increased and ITI significantly decreased after MVF training (* p<0.05; ** p<0.01).*

Finally, when comparing the Δ score of the number of fingers movements, RM-ANOVA showed no significant effect of Hand, Group or Hand *Group interaction (p always >0.05), indicating that performance gain was similar in both hands for both groups (Figure 3).

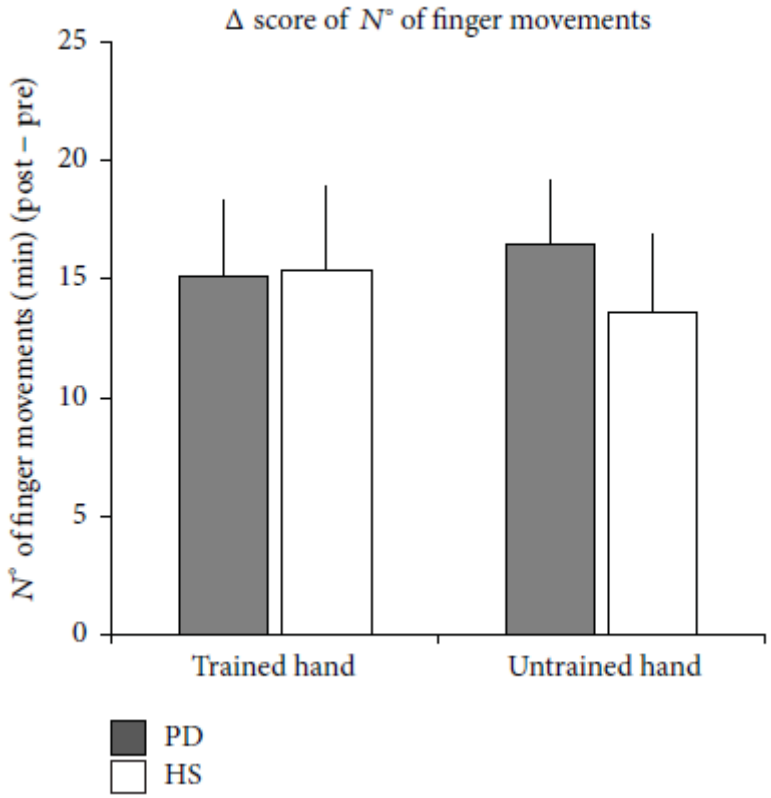

Figure 3. *Behavioural data, showing the Δ score of the numbers of finger movements performed in one minute (number of finger movements/min after MVF training- number of finger movements/min before MVF training) in the trained and untrained hands of both Parkinson's disease (PD) patients (grey bars) and Healthy Subjects (HS) groups (white bars). Vertical bars indicate standard error of the mean (SEM).*

However, when this Δ score was correlated with PFS-16 clinical score, a significant correlation was found only for the trained hand (trained hand: Spearman rho=0.64, p=0.024; untrained hand: Spearman rho=0.54, p=0.07), indicating that the higher the fatigue symptom the lower the performance improvement (Figure 4).

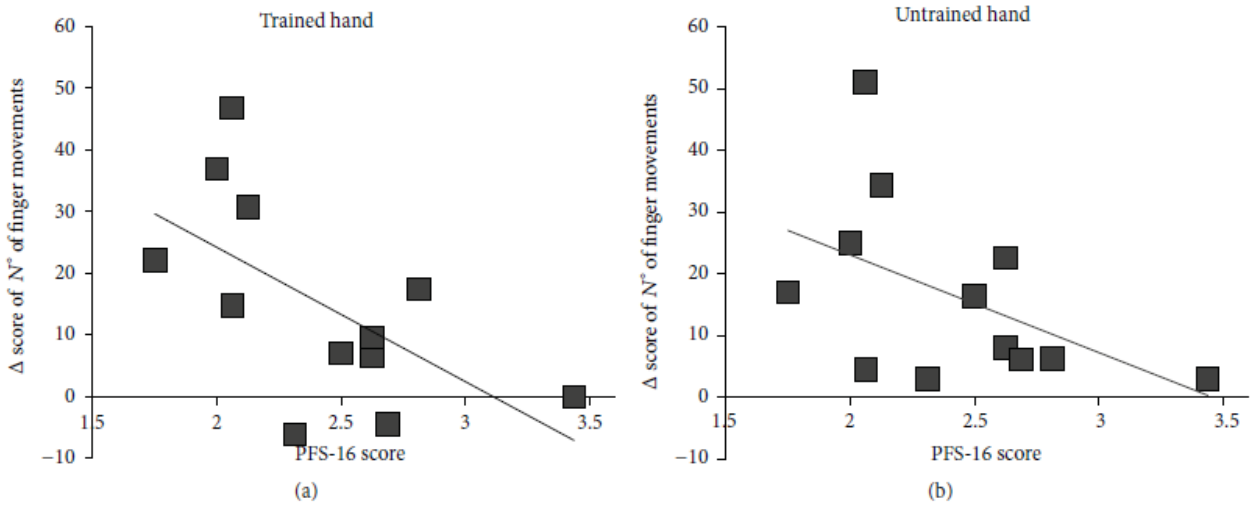

Figure 4. *Correlation analysis between individual changes in the number of finger tapping movements induced by mirror visual feedback practice and individual scores at the Parkinson Fatigue Scale (PFS-16) questionnaire in Parkinson's disease patients. There is a significant positive correlation between the improvement in the less affected/trained hand (panel A) and the clinical score (r= 0.64; p = 0.024), indicating that the higher the fatigability, the lower the performance improvement. In panel B the non-significant correlation between the improvement of the more affected/untrained hand and PFS-16 is depicted (r=0.54, P=0.07).*

Cortical excitability

For the IO curve the RM ANOVA showed a significant effect of Time ($F_{(1,22)}$=14.57, p<0.01) and Intensity ($F_{(4,88)}$= 63.51, p<0.01) (Figure 5). The excitability of each hemisphere, as tested with the IO curve, significantly increased in PD patients and in HS, with no difference between groups ($F_{(1,22)}$ = 0.26, p= 0.61) after the MVF training. The comparison between the trained and untrained hemispheres did not show any significant difference,($F_{(1,22)}$=0.24, p=0.62) demonstrating that cortical excitability in both hemispheres was similarly modified.

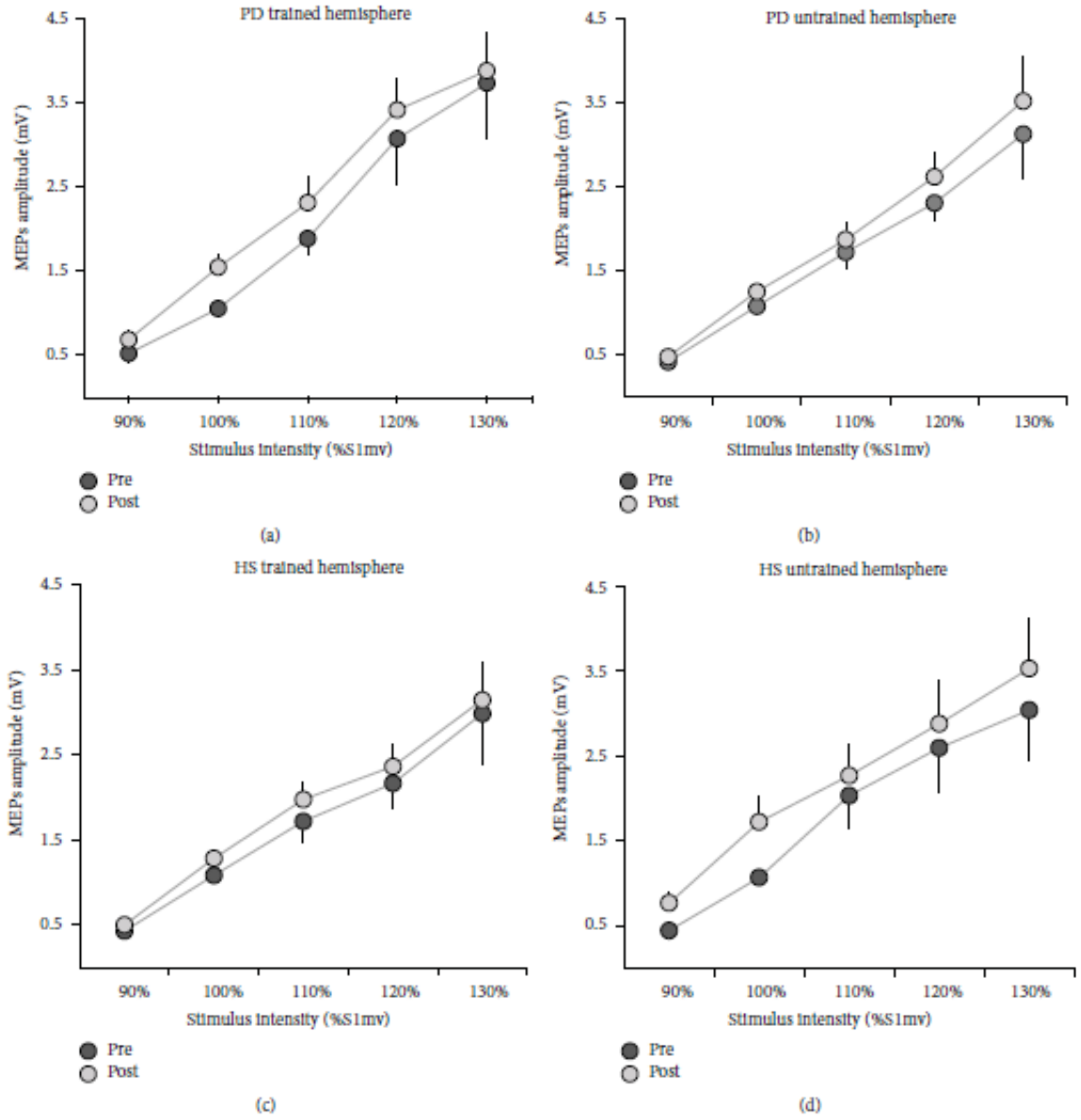

Figure 5. *Input-Output (IO) curves measured in the first dorsal interosseus (FDI) muscle, of the trained (panel **A** and panel **C**) and the untrained (panel **B** and panel **D**) PMCs before and after Mirror Visual Feedback (MVF) training. Data of both groups, Parkinson's disease (PD) patients (panel **A** and panel **B**) and Healthy Subjects (HS) (panel **C** and panel **D**), who underwent MVF training, are shown. MEP amplitudes, in mV, are depicted from 90% to 130% S1mV (the stimulus intensity needed to evoke MEPs of approximately 0.8–1 mV peak-to-peak amplitude). Vertical bars indicate standard error of the mean (SEM).*

Control Experiment

Motor Performance was associated with a larger gain (higher value of Δ score of the number of fingers movements) in the untrained hand in the Mirror PD group with respect to the Control PD group (p=0.041) (Figure 6A). Further, when comparing the IO curves of the untrained hemisphere, RM-ANOVA showed a significant GROUP*TIME interaction ($F_{(1,19)}$=4.17; p=0.035), indicating that the excitability of the untrained hemisphere significantly increased after training only in the Mirror PD group (p=0.012) and not in the Control PD group (p=0.77) (Figure 6B).

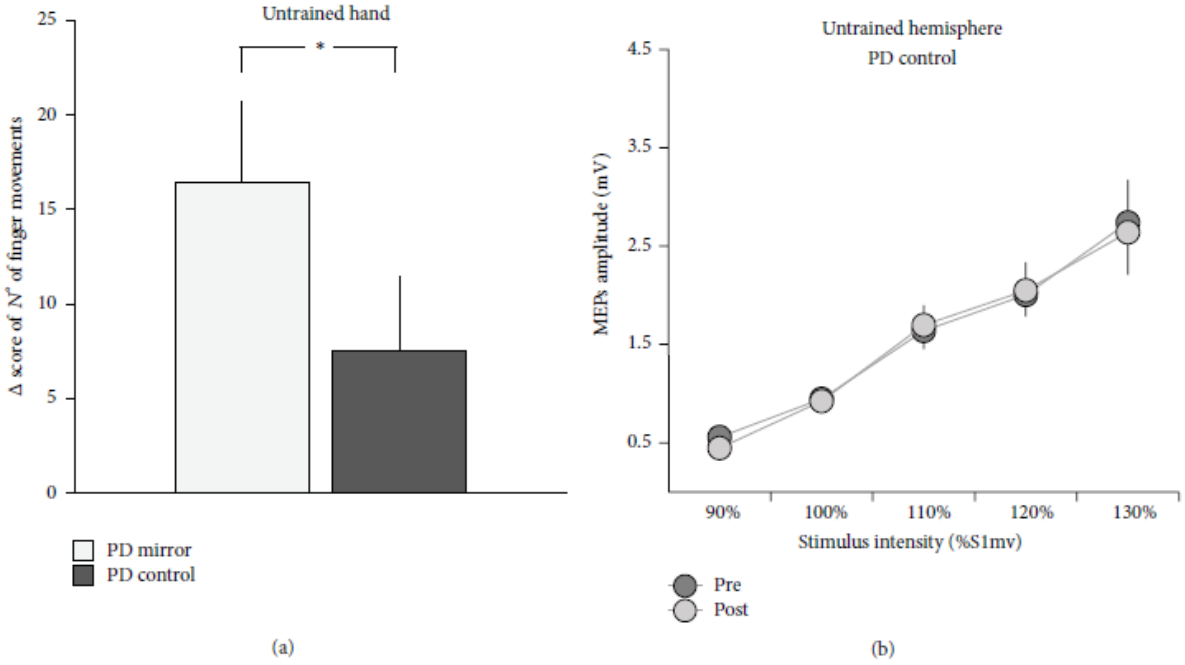

Figure 6. *Data from the Control Experiment (training without MVF). Panel A shows the behavioural data, expressed as performance gain (Δ score of the numbers of finger movements performed in one minute) in the untrained hand of PD patients enrolled in the main experiment (PD mirror) and PD patients enrolled in the control experiment (PD control). In panel B the Input-Output (IO) curves of the untrained PMC of the PD control group, before and after training are depicted. MEP amplitudes, in mV, are depicted from 90% to 130% S1mV (the stimulus intensity needed to evoke MEPs of approximately 0.8−1 mV peak-to-peak amplitude). Vertical bars indicate standard error of the mean (SEM).*

4.1.4 DISCUSSION

The main aim of the present study was to investigate whether unilateral hand training performed by PD patients with the less affected side and based on MVF was able to induce changes in bradykinesia of the untrained (and more affected) hand. Further, we wanted to investigate whether changes in motor performance were accompanied by changes in the excitability of the trained and untrained PMCs. Finally, we wanted to disclose whether the use of MVF might reduce the impact of fatigue on the training- induced improvement of the more affected side in PD patients.

Our main findings were the following: (1) training based on MVF induced an increased number of finger movements, performed in one minute, of the untrained hand in PD patients similarly to HS; (2) this behavioral improvement was associated with the facilitation of excitatory function of the corticospinal pathway, which increased the MEP amplitude in PD patients similarly to HS; (3) the extent of fatigue perceived by PD patients had a greater influence (negative correlation) on the behavioral improvement in the trained hand compared to the untrained one.

The interesting feature of MVF is that only one hand needs to be actively trained to provide performance improvements of both hands[15,17] . Here we took advantage of this feature to establish a proof of evidence on the use of MVF therapy to improve finger movements' bradykinesia in PD. Our PD patients, performed a lower number of finger movements in one minute compared to healthy subjects and this behavior was related to a longer time spent on movement execution (documented by greater inter-tapping interval values). In contrast, the time for the finger touching phase (touch duration), that is the combination of the time used for sensory processing and motor preparation, was not different between PD patients and HS.

After training, all participants increased movement speed by reducing the inter-tapping interval in both the trained and untrained hand and not by changing the touch duration. This finding might suggest that MVF training was able to provide information mainly dealing with the dynamic part of the movement (transition from a finger to the following one in the sequence). This emerging result is in accordance with a previous finding of our group, showing that also when trained with a video showing finger opposition movements (action observation training), PD patients improved bradykinesia by reducing the duration of the time devoted to movement execution[8]. Indeed, a possible mechanism of action of MVF involves the mirror neuron system. In addition to the different cerebral areas involved in the mirror neuron system, the superior temporal gyrus was activated during observation of actions done by others (for a review, see[29–31]) as well as during MVF intervention[32], suggesting a link between MVF and action observation.

Another finding that deserves to be discussed is that in both PD and HS groups the number of correct sequences increased after MVF training in the trained hand but not in the untrained one. Thus, if our findings support the use of MVF therapy to improve slowness of movement execution in PD, MVF does not seem useful to improve spatial accuracy of the untrained hand. One possible explanation may deal with the fact that physical practice and MVF training activates different sensorimotor mechanisms. Physical practice involves both motor and sensory processes[33], as the somatic sensory feedback plays a pivotal role in movement refining[34]. On the other hand, MVF does not supply somatic sensory feedback, but it is based exclusively on a visual feedback. We can suppose that visual information obtained through MVF were able to induce changes only in the dynamic part of movement, similarly to what described for action observation[8].

The last behavioral finding of our study concerns the negative impact of fatigue on bradykinesia improvement that was more evident in the trained hand respect to the untrained one. Fatigue is one of the most disabling non-motor symptoms for people with PD and it has been demonstrated to severely impact quality of life. In this study, we found that the greater was the severity of subjective fatigue (according to PFS-16 score), the less was the improvement in bradykinesia of the trained hand. This finding fits well with evidence in the literature that PD patients show increased physical fatigue during a finger-tapping task and a force decline during a maximum voluntary contraction[35]. Furthermore, although data in the literature are controversial, a relationship between fatigue and the sequence effect[18,36], which represents one of the main features of bradykinesia, has been hypothesized. Our preliminary data may suggest that fatigue can influence the outcome of a training protocol, based on the repetition of sequential movements, aimed to improve bradykinesia. Since no significant correlation was found between fatigue and bradykinesia improvement in the untrained hand, we may suggest the use of MVF as rehabilitative approach in PD patients with fatigue.

It is worth noting that, in addition to behavioural results, the present study showed that PMC cortical excitability was significantly enhanced after MVF training. The Input-Output recruitment curve refers to a global measure of excitability of the corticospinal pathway[37], reflecting not only the number of firing neurons activated by the supra-threshold stimuli, but also the neuronal excitability produced by the sub-threshold stimuli[38]. Robust evidence in the literature obtained in healthy controls already showed that a possible mechanism of action of MVF deals with increased excitability not only of the trained PMC, but also of the untrained one[14,15]. It has already been hypothesized that the increased PMC excitability in the untrained hemisphere might have been caused by the PMC mirror neuron system-like

properties or via dorsolateral prefrontal cortex activation[9,15]. Indeed, MVF creates an intermodal conflict between visual and proprioceptive and tactile senses. The right dorsolateral prefrontal cortex was primarily activated by discrepancies between signals from sensory systems[39]. However, regardless of either mechanism, we found that in PD patients, similarly to HS, the effect of MVF is probably related to the induction of cortical excitability changes in PMC.

Finally, we can reasonably think that performance and excitability changes in the untrained side occurred as a result of MVF. Data from the control experiment in PD patients showed that behavioural changes were greater when training was associated to MVF respect to motor training alone, while the excitability of the untrained hemisphere significantly increased only after MVF training. Indeed, it is worthy to note that learning to perform a motor task with one hand can result in performance improvements in the other hand, a process called intermanual transfer[40–42]. Our results are in line with a recent study[43] showing that, although motor performance significantly increased in the untrained hand in both conditions (with and without MVF), the overall improvement was greater in the mirror group respect to the control group. Our hypothesis is that MVF-associated improvement may derive from the combination of performance gain induced by intermanual transfer (likely via inter-hemispheric mechanisms) *plus* performance gain induced by visual feedback (likely via action observation mechanisms).

In accordance to this hypothesis we showed that training based on MVF may influence the excitability of the transcallosal pathway similarly to training without MVF[16]. Further, we can suppose that action observation mechanisms are involved only during training with MVF since observational learning has been demonstrated to be highly effector-dependent[44]. It

has been shown that finger sequence learning based on observation of right hand performance did not transfer to the left hand[44].

There are some study limitations that should be acknowledged. First, the observed positive effect of MVF training was obtained in a relatively small sample that is not necessarily representative of the whole PD population. Second, in this pilot study, we tested the effect of a single training session in improving finger movements; a longer period of training should be examined. Third, our experimental protocol was designed to study immediate changes of MVF training and we did not assess long-lasting effects.

Conclusions

In this proof-of-concept study, we have provided novel evidence that MVF training might induce improvement in finger movements' bradykinesia of the more affected (untrained) hand in PD patients. We have also shown that the final common pathway for the effect of MVF was the change of excitability in PMC. Further, our findings support the idea that fatigue could impact behavioural improvement in the trained hand more than in the untrained one even if our data have been obtained in a relatively small sample. However, if true, this finding may be relevant for future clinical studies that aim to improve bradykinesia in PD patients suffering from fatigue.

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CHAPTER 5. PhD ACTIVITIES

5.1 PUBLICATIONS DURING PhD PERIOD RELATED TO THIS THESIS

Bonassi G, Biggio M, Bisio A, Ruggeri P, Bove M, Avanzino L. Provision of somatosensory inputs during motor imagery enhances learning-induced plasticity in human motor cortex. Sci Rep. 2017 Aug 24;7(1):9300.

Bonassi G, Pelosin E, Ogliastro C, Cerulli C, Abbruzzese G, Avanzino L. Mirror Visual Feedback to Improve Bradykinesia in Parkinson's Disease. Neural Plast. 2016;2016:8764238.

5.2 OTHER PUBLICATIONS DURING PhD PERIOD

Avanzino L, Ravaschio A, Lagravinese G, **Bonassi G**, Abbruzzese G, Pelosin E. Adaptation of feedforward movement control is abnormal in patients with cervical dystonia and tremor. Clin Neurophysiol. 2018 Jan;129(1):319-326.

Pelosin E, Ogliastro C, Lagravinese G, **Bonassi G**, Mirelman A, Hausdorff JM, Abbruzzese G, Avanzino L. Attentional Control of Gait and Falls: Is Cholinergic Dysfunction a Common Substrate in the Elderly and Parkinson's Disease? Front Aging Neurosci. 2016 May 9;8:104.

Lagravinese G, Pelosin E, **Bonassi G**, Carbone F, Abbruzzese G, Avanzino L. Gait initiation is influenced by emotion processing in Parkinson's disease patients. Movement Disorders, *in press.*

Pelosin E, Cerulli C, Ogliastro C, Lagravinese G, Mori L, **Bonassi G**, Mirelman A, Hausdorff JM, Abbruzzese G, Avanzino L. Interplay between cholinergic function and obstacle negotiation:

effect of V-TIME training in elderly and Parkinson's disease patients at fall risk. *Submitted* to Neurorehabilitation & Neural Repair

5.3 ABSTRACT SUBMITTED TO NATIONAL AND INTERNATIONAL CONFERENCES DURING PhD PERIOD

Bonassi G, Ravaschio A, Pelosin E, Lagravinese G, Marchese R, Abbruzzese G and Avanzino L: Anticipatory behavior and adaptation during catching a ball in patients with cervical dystonia. I Congresso Accademia Limpe-Dismov, Torino 2015

Avanzino L, Pelosin E, **Bonassi G**, Ogliastro C, Ruggeri P and Bove M. A peripheral sensory stimulus modulates the excitability of cerebello-cortical connectivity. 66th SIF National Congress. Genova 2015

Bonassi G, Ogliastro C, Lagravinese G, Pelosin E, Abbruzzese G and Avanzino L: Mirror Visual Feedback to improve bradykinesia in Parkinson's disease. II Congresso Accademia Limpe-Dismov. Bari 2016.

Lagravinese G, Avanzino L, Ravaschio A, **Bonassi G**, Pelosin E, Abbruzzese G. Gait initiation in response to emotion-inducing pictures in Parkinson's disease. II Congresso Accademia Limpe-Dismov. Bari 2016.

Bonassi G, Biggio M, Bisio A, Bove M, Ruggeri P, Avanzino L. Motor cortical plasticity induced by motor learning through motor imagery combined with peripheral nerve electrical stimulation. 4th International Workshop on Synaptic Plasticity: from bench to bedside, Fresco Conference, Lucca, 2016

Lagravinese G, **Bonassi G**, Ravaschio A, Pelosin E, Marchese R, Abbruzzese G and Avanzino L. Anticipatory behaviour and motor adaption during catching a ball in patients with cervical dystonia. XXIV Congresso Nazionale della SIPF, Milano 2016.

Bonassi G, Bisio A, Lagravinese G, Pelosin E, Carbone F, Bove M, Avanzino L. Sensorimotor modulation during motor imagery. III Congresso Accademia Limpe-Dismov. Verona 2017

Carbone F, Pelosin E, Ogliastro C, Lagravinese G, Putzolu M, Crisafulli O, **Bonassi G**, Abbruzzese G and Avanzino L. Addition of non-immersive virtual reality to treadmill training drives changes in cholinergic activity and obstacle negotiation in patients with Parkinson's disease and older adults. III Congresso Accademia Limpe-Dismov. Verona 2017

Bonassi G, Pelosin E, Ogliastro C, Lagravinese G, Mirelman A, Hausdorff J.M., Abbruzzese G and Avanzino L. Cholinergic dysfunction: a common substrate for attentional control of gait among fallers in older adults and people affected by Parkinson's disease. I Congresso Accademia Limpe-Dismov , Torino 2015.

5.4 PARTICIPATION TO SUMMER SCHOOLS AND TRAINING

From June 22, 2015 to June 26, 2015
Summer school "Integrazione di metodi e tecniche per la ricerca, la clinica e la riabilitazione in psicofisiologia e neuroscienze."

From October 8, 2016 to October 10, 2016
Movement Disorders Society Physiotherapy Summer School, The Leuven Institute for Ireland in Europe, Leuven, Belgium

From September 4, 2017 to September 29, 2017
EEG training at the Research center for motor control and neuroplasticity, KU Leuven, Leuven, Belgium under the supervision of Prof. Dante Mantini

List of Abbreviations

ADM	Abductor Digiti Minimi
CB	Cerebellum
EMG	Electromyographic signal
ES	Peripheral Nerve Electrical Stimulation
ESMI	Electrical Stimulation and Motor Imagery
FDI	First Dorsal Interosseus
fMRI	Functional MRI
GAS	Glove Analyzer System
HS	Healthy Subject
ITI	Inter Tapping Interval
KVIQ	Kinaesthetic and Visual Imagery Questionnaire
LTD	Long Term Depression
LTP	Long Term Potentiation
ME	Movement Execution
MEP	Motor Evoked Potential
Metr	Metronome
MI	Motor Imagery
MIQ	Movement Imagery Questionnaire
MR	Movement Rate
MS	Maximum Speed
MVF	Mirror Visual Feedback
NIBS	Non Invasive Brain Stimulation
PAS	Paired Associative Stimulation
PD	Parkinson's Disease
PET	Positron Emission Tomography
PMC	Primary Motor Cortex
PP	Physical Practice
PPC	Posterior Parietal Cortex
SAI	Short Afferent Inhibition
SD	Standard Deviation
SE	Standard Error
SMA	Supplementary Motor Area
SPM	Self Paced Mode
TACS	Transcranial Alternate Current Stimulation
TD	Touch Duration
TDCS	Transcranial Direct Current Stimulation
TE	Timing Error
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation